STUDY OF THE EFFECT OF GA_3 APPLICATION ON THE PERFORMANCE OF SUNFLOWER

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Dedicated

To

My Loving Parents

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Certificate

This is to certify that the dissertation entitled, **"Study of the Effect of GA_3 Application on the Performance of Sunflower"** submitted in partial fulfilment of the requirements for the degree of Master of Philosophy in Botany, is a faithful record of the bonafide research work carried out at the Aligarh Muslim University, Aligarh by **Ms. Nikhat Jafri** under my guidance and supervision and that no part of it has been submitted for any other degree or diploma.

(Firoz Mohammad)
Research Supervisor

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Nikhat Jafri

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CONTENTS

Introduction

CHAPTER 1

INTRODUCTION

Oilseeds occupy an important position in Indian agriculture being next to food grains as a farm commodity. The important oilseeds produced in the country include castor seed, groundnut, linseed, niger seed, rapeseed-mustard, safflower seed, sesame seed, soyabean and sunflower seed. Of these, castor seed and linseed are the source of non-edible oils basically for industrial use. Edible oils are used as such or after hydrogenation mainly for cooking purposes.

Sunflower (*Helianthus annuus* L.) is one of the fastest growing oilseed crops of India. It was introduced as an oilseed crop in 1969. This crop has gained importance due to its short duration of maturity, containing of excellent quality of oil, photo-insensitivity, wide adaptability into different kinds of cropping pattern, high energy hull and drought tolerance. The commercial cultivation of sunflower began in early seventies with a meagre area of 15 thousand hectares. It has gone up to 2.07 million hectares of the area with a production of 1.25 million tonnes in the year 2004. As for its place among oilseed crops, it occupies the fourth place in terms of acreage and production. Sunflower is the major non-conventional oilseed corp. It has been described as "drenched with sun-vitality" because the head follows the sun, ending up facing the west "to absorb the few last rays for the dying sun" (Nagaraj, 1995; www.agmarknet.nic.in; www.fao.org). Moreover, sunflower has the potential to produce the highest oil yield per hectare and is also a good source of honey

(Munir, 2006). Therefore, it is highly desirable to supplement our oilseeds production through the cultivation of sunflower, as it contributes 23.68% of the domestic edible oil production and may substitute imports substantially (Anonymous, 2006).

Sunflower seeds contain 48-53% oil. The oil is generally considered as a premium oil because of its light colour, higher level of unsaturated fatty acids and high smoke points. The oil contains 90% unsaturated fatty acids as oleic and linoleic acids with the remainder consisting of palmitic and stearic saturated fatty acids. Its consumption reduces the level of blood cholesterol, a factor which is responsible for the incidence of coronary heart disease (Munir, 2006; Kalaiyarasan and Vaiyapuri, 2007; www.agmarknet.nic.in). The oil is also rich in vitamin A, D, E and K, essential for health (Pandey, 2000). Moreover, the occurrence of alfatoxins in the seeds is rare. The oil cake left after the extraction of the oil is rich in high quality protein (40-44%) and is used as cattle and poultry feed.

However, according to FAO (2004), the average production of seeds in our country is low (603 kg/ha) compared with the world average of 1225 kg/ha and also non-availability of quality seeds for further seed production (Uppar and Kulkarni, 1989; Khan *et al.*, 2003; www.fao.org). The situation necessitates to find out ways to increase productivity on sustained basis. It may be added that efforts have been made to boost up the productivity not only by adopting the scientific agro-practices but also by overcoming the incomplete development of seeds, among other shortcomings.

Due to the high priority accorded to foodgrains, not much can be done to bring more land under oilseed cultivation. Moreover, a majority of farmers (75%) has marginal holdings of less than two hectares. Keeping in mind such a limitation on increasing the acreage for cultivation, it is highly desirable to innovate ways which can augment the yields. One such approach could be to facilitate the utilization of the available resources leading to maximum harvesting of solar energy and subsequently increasing the number of active sinks. To achieve this, plant growth regulators could be used as they are known to affect many facets of plant life including growth, flowering, fruiting and ion transport (Wareing and Phillips, 1981; Khan *et al.*, 2002; Khan and Samiullah, 2003; Siddiqui and Mohammad, 2003).

Gibberellins are known to control a wide range of physiological functions in plants. For example, application of gibberellic acid (GA_3) improves cell elongation and cell differentiation. Therefore, the present author proposed to apply GA_3 to sunflower seeds to increase plant growth for better harvesting of solar energy.

It was, therefore, decided to undertake a factorial randomized pot experiment with the following objective in mind:

To establish the best concentration of GA_3 for pre-sowing seed treatment and to determine the most effective soaking duration for the optimum performance of PAC 3776, a locally popular cultivar of sunflower.

The details of the experiment are given in Chapter 3 (pp. 21-37).

Review of Literature

CONTENTS

CHAPTER 2

REVIEW OF LITERATURE

Farmers have been growing crop plants for a long time. However, their production has failed to keep pace with ever increasing demand and thus there is always a need for improvement in their productivity. Farm scientists have been able to demonstrate that the productivity of crop plants could be improved to a great extent through proper selection of cultivars, balanced mineral nutrition, adequate plant protection measures, improved agronomic practices, adequate internal hormonal balance and proper partitioning between source and sink, among others. In the following pages, an effort has been made to review the available literature on the general aspects of sunflower, on plant growth regulators and on crop response to exogenous application of GA_3.

2.1 Sunflower

Sunflower is popularly known as 'surajmukhi' as it follows the sun by day, always turning towards its direct rays. It belongs to the genus *Helianthus* which has been derived from 'Helios' meaning sun and 'anthos' meaning flower. The genus belongs to the family Asteraceae.

2.1.1 Botanical description

It is an annual, erect and herbaceous plant with leaves simple, alternate with stout petioles and lanceolate shape. Leaves are rough on both surfaces. Plants have a composite inflorescence, referred to as capitulum or head. The capitulum consists of a receptacle with involucres bracts that are modified

leaves; ray-flowers on the outer whorl of the receptacle that are sterile and golden yellow, but may be pale yellow, orange yellow or reddish; disk-flowers on the inner whorl of the receptacle that are perfect flowers of yellow or brown colour. The cultivated genotypes are characterized by a single stem terminating in a capitulum. Sunflower is protandrous, in which the male and female elements mature at different times. There appears to be a time-lag of 18-24 hours in the maturity of the male and female elements. So it is essentially a cross pollinated plant, besides showing varying degrees of self-incompatibility. The flowers are pentamerous and epigynous. The corolla is of five fused petals. The stamens are syngenesious. The filaments are free but anthers are usually connate into a tube around the style. The anthers are dithecous, introrse and opening by the longitudinal slits. Gynoecium is bicarpellary and syncarpus. The ovary is inferior and unilocular with a single basal anatropous ovule and basal placentation. Fruit is cypsela, a single head produces 350 to 2000 seeds. Seeds are pointed at base and round at end. Colour of the seeds varies from black to white but brown, striped or mottled seeds may also occur (Weiss, 1983; Sharma, 2000; Singh and Jain, 2001; Reddi and Reddy, 2002).

2.1.2 Classification

According to the system of classification given by Bentham and Hooker (1862-1883), the aforesaid oil producing species could be classified as follows:

Kingdom	-	Plant Kingdom
Division	-	Phanerogamia
Sub-division	-	Angospermae
Class	-	Dicotyledons
Sub-class	-	Gamopetalae

Series	-	Inferae
Order	-	Asterales
Family	-	Compositae
Genus	-	*Helianthus*
Species	-	*Helianthus annuus* L.

However, it may be added that the current name of the family is Asteraceae (Cronquist, 1981) and the classification given by him is as follows :

Kingdom	-	Plantae
Sub-kingdom	-	Tracheobionta
Sub-division	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Sub-class	-	Asteridae
Order	-	Asterales
Family	-	Asteraceae
Genus	-	*Helianthus*
Species	-	*Helianthus annuus* L.

2.1.3 Origin

Sunflower is probably originated in southern United States and Mexico from where it was introduced into Europe and later into former USSR. It was taken to Spain before the middle of the sixteenth century. In the nineteenth century, the cultivation of sunflower as an oilseed crop began in the Soviet Union and the majority of the present day varieties grown all over the world trace back their origin to the USSR (Weiss, 1983; Reddi and Reddy, 2002).

2.1.4 Distribution

Sunflower is grown in many countries of the world including Argentina, Bulgaria, Canada, Rumania, Russia, Turkey and South America. In India, it

was introduced in 1969 and became quite popular among the farmers. At present, it is grown extensively in Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu (Weiss, 1983; Pandey, 2000; Anonymous, 2002; Reddi and Reddy, 2002).

2.1.5 Climate and soil

This crop requires a cool climate during germination and seedling growth, warm weather from the seedling stage up to flowering and non-cloudy sunny days during flowering to maturity. The crop can thrive well in a variety of soils. It performs well in deep, natural and well-drained light soils as well as in heavy soils. The optimum pH of the soil for this crop is 6.5 to 8.5 (Anonymous, 2002; Reddi and Reddy, 2002).

2.1.6 Cultivation

For sunflower, the season of planting, the photoperiod and within limits the altitude are not the limiting factors. Hence it is possible to cultivate this crop throughout the year. At the time of sowing, the soil should be friable and free from weeds. Three to four ploughings and diskings are sufficient for preparing the land. Soil should be moist at least the depth of about 10 cm before sowing and this condition necessitates good soaking rains or irrigation before sowing. Well-filled plump seeds at 8 to 10 kg are required to cover one hectare. For controlling seed-borne fungal diseases, seed treatment with either 'brassical' or 'capton' at the rate of 3 g/kg of seed is recommended. The seeds are drilled at a depth of 5 cm by adopting a spacing of 45 cm between rows and 30 cm between plants in the row. The population densities recommended are 60,000-80,000 plants per hectare under irrigated conditions. A basal dose of 40

kg nitrogen (N), 26 kg phosphorus (P) and 17 kg potassium (K) per hectare is recommended under irrigated conditions. Generally, two hoeings are sufficient for the successful cultivation of this crop (Weiss, 1983; Anonymous, 2002; Reddi and Reddy, 2002).

2.1.7 Harvesting and threshing

The sunflower crop matures in 90-100 days. The crop has to be harvested when the lower side of the head turns green to lemon yellow colour and some of the bracts dry up. At physiological maturity, the seeds attain maximum weight and oil concentration and harvesting at this stage results in highest seed and oil yield. Ten per cent of heads should turn brown and florets attached to the tip of the seeds drop off naturally. During harvesting, proper care should be taken to avoid quantitative and qualitative losses. The harvesting of the crop is done by means of hand operated sickles. The crop is made into bundles and stacked in the sun for a couple of days. Then it is threshed by beating the seed bearing parts of the plants taken in convenient sized bundles, by means of a wooden mallet to separate the seeds. The cleaned and threshed seeds may then be dried in the sun for another couple of days and then stored in seed bins or gunny bags. The storage room should be completely free from humidity (Weiss, 1983; Reddi and Reddy, 2002).

2.1.8 Uses

In India, sunflower was used mainly as ornamental crop but in recent past it became an important source of edible and nutritious oil. It is a major source of vegetable oil in the world. Its seeds contain about 48-53% edible oil. The oil is light yellow in colour. It possesses good flavour and high smoking

point. The oil is easily digestible. The oil is rich source (64%) of linoleic acid which is good for heart patients. Linoleic acid helps in washing out cholesterol deposition in the coronary arteries of the heart. The oil is free from heart disease causing linolenic acid, erucic acids and cholesterol. The oil is also used for manufacturing hydrogenated oil. Sunflower oil contains protein, vitamins A, D and E. Being of semidrying and stable type, sunflower oil is used in making paint, varnish and soap. It is also used in the preparation of cosmetics and pharmaceuticals. Oilcake is the byproduct of the sunflower oil extraction and is a source of protein for animal feed blends. Sunflower oilcake, however, is considered to be of relatively poor quality due to high crude fibre content. Sunflower seeds make a nutritious food for cattle, poultry, hogs and cage birds (Nagaraj, 1995; Pandey, 2000; Anonymous, 2002; Reddi and Reddy, 2002).

2.2 Phytohormones

There are numerous substances natural and synthetic that have profound influence on growth and differentiation of plant cells and organs. Their role in development has been studied for nearly a century, yet the concept of hormones in plants is steeped in controversy. In 1905, the British physician E.H. Starling introduced the term hormone to describe these chemical messengers (Hopkins, 1999).

The term phytohormone was coined by Thimann in 1948 who defined it as "an organic compound produced naturally in higher plants controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts" (Sinha, 2004).

The exact location of synthesis of phytohormones is uncertain but actively growing tissues, leaves, developing seeds thought to be active sites of synthesis of phytohormones. However, it appears that all tissues have the potential to produce only of the phytohormones, which are transported via xylem or phloem (Weiler and Ziegher, 1981). The prevailing direction of transport depends on the type of phytohormone and development stage of plant. Phytohormones act at genetic level (Bajguz, 2000; Marschner, 2002; Taiz and Zeiger, 2006). The commonly recognized classes of plant hormones are auxins, gibberellins, cytokinins, abscisic acid, ethylene and are now supplemented with brassinosteroids and jasmonic acid (Dewitt and Ockelen, 2001).

2.2.1 Gibberellins

Gibberellins are chemically closely related to diterpens, which are themselves members of vast group of naturally occurring compounds in plants called terpenoids. The discovery of gibberellins dates from 1898, when Korishi, for the first time described "bakanae disease" (foolish seedling) of rice with characteristics symptoms of tall spindy plants (Arteca, 1996). In 1926, Kurosawa, for the first time reported gibberellin from the cell culture of *Gibberella fujikuroi*. In 1938, Yabuta and Sumiki were successful in isolating a small quantity of high active crystalline material from sterile culture filtrates and was given the name "gibberellin A" as it was isolated from *Gibberella*. Subsequently they isolated another compound of similar nature and named it "gibberellin B". In 1954, British chemists Brian and others identified and chemically characterized a pure compound from culture filtrates of *Gibberella fujikuroi*. They called this new substance "gibberellic acid" (Fig. 1). Two teams, Brian *et al.* in 1954 at the Imperical Chemical Institute (ICI) in England

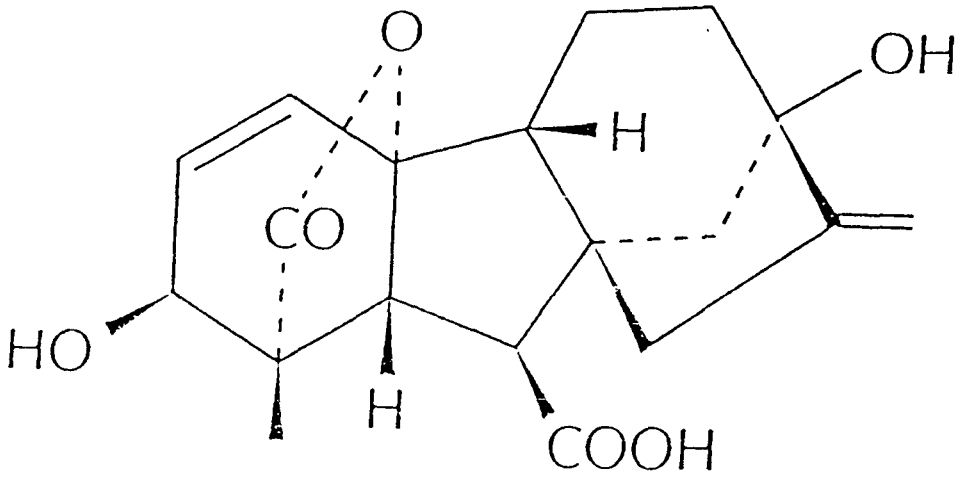

Fig. 1 . Structure of gibberellic acid

and Stodola *et al.* in 1955 at the U.S. Department of Agriculture (USDA), working on large scale preparation of gibberellins from the fungus culture, isolated entirely new compound. The ICI team gave the name "gibberellic acid" while the USDA team, "gibberellin X". The former name has been universally accepted and gibberellic acid is now also known as GA_3 (Moore, 1989). At present, the number of gibberellins known from all sources, including plants is 125. They differ from one another by the presence or absence of the location configuration (internal ester) in the ring A and the substituents, mainly hydroxyl groups about the whole ring structure. Due to presence of an additional ethylenic double bond in ring a, GA_3 is more unsaturated and, there by, more active than other gibberellins (Salisbury and Ross, 1992; Buchanan *et al.*, 2000; Kumar and Purohit, 2003; Sinha, 2004; Singh, 2005).

Exogenous application of GA_3 has been shown to relieve certain type of dormancy including physiological dormancy, photodormancy and thermodormancy (Hartmann *et al.*, 1990) and to promote flowering in a variety of plant species under non-inductive conditions (Zeevaart, 1983; Harkness and Lyons, 1994). The influence of gibberellins includes parthenocarpic fruit development, senescence, promote cell growth, increase cell wall plasticity, stem elongation and growth of whole plant, among others (Salisbury and Ross, 1992; Taiz and Zeiger, 2006).

2.3 Methods of phytohormone application

In nature, phytohormone required for growth and development are synthesized in plants themselves. However, they could be added exogenously

to exploit the full genetic potential of plants. Generally, the hormones are supplied to plants via pre-sowing seed treatment or through foliar application as dilute solutions at crucial stages (Ahmad *et al.*, 2001; Hayat *et al.*, 2001; Khan and Samiullah, 2003; Afroz, 2006).

2.4 Response of sunflower to phytohormone application

A lot of work has been done on the effect of phytohormones on the performance of oilseeds of the family Asteraceae. In the following pages, an effort has been made to review the available literature on the sunflower and safflower for the last three decades.

De-La-Guardia and Benlloch (1980) performing an experiment on sunflower noted that application of 10 µl GA_3 solution (100 µg/ml GA_3 in 0.05%, v/v, Tween 20) on each cotyledons of 6 day (d) old seedlings resulted in a tenfold increase in the length of the first internode. They also noted an increase in the content of reducing sugars.

Umoessien and Forward (1982) studied the effect of GA_3 on the distribution of product of photosynthesis in sunflower. GA_3 was applied to the same leaf or to the terminal bud or the roots, and the distribution of assimilated ^{14}C was determined at intervals of 1-96 h. GA_3 had no significant effect on initial distribution of ^{14}C during the period of rapid export from the leaf, but enhanced re-export from the root after translocation from the leaf had virtually ceased. Most of the ^{14}C exported from the roots accumulated in the shoot tip. The site of application of the hormone was of relatively minor importance. Wherever it was applied the major effect was enhancement of movement from the roots to the shoot tip. Application to the terminal bud was most effective in

this respect. There was no evidence that GA_3 directly affected the transport system, but the data support the hypothesis that it increases the strength of the sink in the shoot tip.

Shukla *et al.* (1987) studied the effect of GA_3 on seed setting and seed filling in sunflower. 200 ppm GA_3 was applied to the buds of sunflower cvs. EC 68413 and EC 68414 at the opening stage, 45 days after sowing (DAS). Control plants were treated with distilled water. At maturity, heads were divided into 4 equal parts across the centre and seeds were collected from peripheral, middle and central portions of each part. Hollow seeds were separated from filled seeds. Total number of seeds/100 cm^2 was greatest in the central portion in both cultivars. The GA_3 treated plants had higher number of seeds in all 3 portions of both cultivars than the control plants.

Czapla *et al.* (1988) conducted trials to investigate the effect of applying GA_3, indole acetic acid (IAA), kinetin (Kn) and phenylacetic acid (PAA) on growth and development of sunflower. Sunflowers were given foliar sprays of 3 mg GA_3 or Kn or 2 mg IAA/dm^2 or a mixture of all three, or GA_3, Kn or PAA applied directly to the soil or as an oil emulsion covering urea granules. Growth regulator application method did not significantly affect fresh weight or height, although foliar GA_3 applications tended to be most effective. Applying growth regulators with urea was most effective in increasing inflorescence number.

Al-Gharbi and Yousif (1989) conducting an experiment on sunflower, noted that applied GA_3 increased seed protein content whereas of chlormequat increased seed oil content.

Kene *et al.* (1991) conducting an experiment on sunflower cv. G.V. EC 68414, studied the effect of foliar application of growth regulators on growth, yield and oil content. Data revealed that, the seed yield was significantly increased with GA_3 at 15 and 30 ppm and indole butaric acid (IBA) at 30 ppm sprayed at flowering stages. Similar trend was also noticed in respect of plant height, leaf area index (LAI), head diameter and oil content.

Pearce *et al.* (1991) performing an experiment on sunflower cv. Delgren 131, noted that the treatment with GA_3 or GA_1 applied to the cotyledonary petioles of 6 d old seedlings results in faster elongation of hypocotyls.

Beltrano *et al.* (1994) conducting an experiment on sunflower cv. SPS 894 and ACA 882, studied that the application of foliar spray of GA_3 (150 mg/l) at 20, 40 or 60 days after emergence (DAE) did not affect yield components. However, foliar spray of benzyl adenine (BA) at 150 or 250 mg/l with or without GA_3 particularly at 40 or 60 DAE reduced the percentage of empty echenes and increased achene weight, 1000-achene weight and achene number and seed yield.

Kene *et al.* (1995) performing an experiment on sunflower cv. EC 68414, noted that the spray of 50 or 30 ppm GA_3 or 30 ppm IBA increased plant height, LAI, head diameter, seed yield and oil content.

Almeida and Pereira (1996) studied the involvement of GA_3 in the control of flowering of sunflower cv. 33 in an experiment by direct application of GA_3 to the apex of the plants, analysis of the endogenous levels of gibberellin like substances at different plant ages, and indirectly by the application of paclobutrazol, an inhibitor of gibberellin synthesis. Aqueous

solution of GA_3 (10^{-3}M) was applied as a 30 µl droplet to the apices of the plants with the help of a graduated microsyringe, with GA_3 applying every two day from 10^{th} to 20^{th} d. GA_3 speeded up flower initiation and floral apex development. The time of GA_3 application was more critical than the amount of GA_3 applied. The application of paclobutrazol markly delayed floral initiation and this effect was also dependent on plant age. Both GA_3 and paclobutrazol had their greatest effects between 10 and 20 DAS suggesting that an increase in GA_3 in that time period plays a role in floral initiation.

Almeida and Pereira (1997) conducted an experiment to investigate the effect of GA_3 and paclobutrazol on vegetative development of sunflower cv. 33. They applied 5-30 µl of 10^{-3}M GA_3 to the apices of 10 d old seedlings or 30 µl to the apices of 10 to 20 d old seedlings or immersed some seeds in GA_3 for 24 h before sowing. In a second set of experiments, 10^{-3}M paclobutazol was applied in 20 µl drops to apices of 10-20 d old seedlings or to the soil of 10-14 d old seedlings. The stimulatory effect of GA_3 on plant height was dependent on age of seedlings, the younger plants being most sensitive. Paclobutrazol when applied to the soil caused dwarfing and retarded leaf expansion, the younger seedlings being more sensitive.

Hernandez (1997) studied the effect of exogenous application of plant growth regulators on morphogenesis of sunflower cv. Dekalb G100. The plants were given 45 µg naphthalene acetic acid (NAA) or BA/plant/d for 10 days or 45 µg GA_3/plant/d for 5 days from the commencement of capitulum development. Growth regulators were injected into unfolded leaves of the terminal bud. NAA had no significant effect on development compared with

untreated controls. GA_3 increased the length of stem internodes and accelerated the onset of floral development by 25%. The most effective growth regulator was BA, which increased leaf area by 38%, stem dry weight by 93% and significantly changed capitulum morphology with an increase in the number of floret primordia of 17% as a result of increased expansion of the receptacle before onset of floret differentiation.

Baydar (2000) studied the effect of GA_3 application on male sterility seed yield, oil content and fatty acid synthesis of safflower (*Carthamus tinctorius* L.) cv. Yanice 5-38. GA_3 was applied at 0, 50, 100, 200 or 300 ppm at 40, 55 and 70 DAS. GA_3 induced male sterility at rates of up to 93%, and decreased seed yield per plant. Although GA_3 did not affect fatty acid synthesis, oil synthesis increased with increasing GA_3 concentration from 33.8% in controls to 38.8% with the application of 300 ppm at the budding stage.

Shankar *et al.* (2000) studied the effect of pre-harvest spray application of $CaCl_2$ (0.1, 0.5 and 1.0%). BA (10, 20 and 30 ppm) and GA_3 (50, 100 and 150 ppm) along with water as the control at 60, 70 or 80 DAS on the seed quality of sunflower cv. Morden. Sprays of plant growth regulators were given on capitulum. Application of 100 ppm GA_3, especially at 60 DAS and storage of achenes, particularly in poly pack, were more effective in maintaining seed quality in the terms of seedling vigour index.

Dholekar *et al.* (2001) studied the effect foliar sprays of four growth regulators, viz. GA_3, succinic acid (SA), 2,3,5-triiodo benzoic acid (TIBA) and Kn on yield and yield attributes of safflower cv. Bhima. Control comprised

without any spray of growth regulator. GA_3 was applied at 50 ppm, SA at 1%, TIBA at 500 ppm and Kn at 10 ppm at 20 or 30 DAS. Application of Kn at 20 DAS was found significantly superior for seed yield and other characters (number of branches per plant, number of capitula per plant and number of seeds per capitula). Seed oil content was significant highest with Kn applied at 30 DAS stage. TIBA applied at both stages although inhibited stem elongation showed significant increase in yield and yield contributing characters. The spray of GA_3 and SA applied at 30 DAS stage occupied second and third position in respect of seed yield.

Shanker *et al.* (2001) studied the effect of sprays of $CaCl_2$ (0.1, 0.5 and 1.0%), BA (10, 20 and 30 ppm) and GA_3 (50, 100 and 150 ppm) at 60, 70 or 80 DAS on growth and yield characteristics of sunflower cv. Morden. They noted that foliar application of GA_3 followed by $CaCl_2$ and BA particularly at 60 DAS significantly increased the total dry matter production. They further noted that sprays of BA (30 ppm) and $CaCl_2$ (0.5%) increased the capitulum diameter, test weight, yield and oil content.

Baydar (2002) studied the effect of foliar spray of GA_3 on the performance of safflower cv. Dincer 5-118. He applied four concentration of GA_3 (100, 200, 3000 and 400 ppm GA_3) on buds at 75 DAS. Exogenously applied GA_3 decreased the levels of IAA and ABA. The lowered endogenous GA_3/ABA and Zeatin/IAA ratios in the seeds significantly decreased the germination percentage and hypocotyls elongation, respectively. The seeds from GA_3 treated plants had more hull percentage and less oil content than seeds from the non GA_3 treated plants. As a consequence, it was indicated that

poor germination and emergence vigour might be a major problem in hybrid safflower seeds produced from plants treated with GA_3.

Cecconi *et al.* (2002) studied the effect of spray of GA_3 on the stem elongation of a dwarf mutant *dw1* of sunflower. They applied 20 ml of GA_3 at 0, 0.01, 0.1, 1, 10 and 100 ppm weekly till flowering or till 2, 4, 6 and 8 weeks. They reported that periodic treatment with GA_3 was effective to revent to the wild type phenotype and internode elongation was directly related to the GA_3 concentration.

Vasudevan *et al.* (2002) studied the effect of growth regulators on seed yield, yield parameters and oil content of sunflower genotypes. They applied spray of (i) TIBA at 240 ppm, (ii) TIBA at 240 ppm + NAA at 50 ppm (iii) 50 ml mixture of IAA (1 ppm) + GA_3 (5 ppm) + cytokinin (0.1 ppm) in 200 l water, (iv) 100 ml of the mixture (iii above), (v) 100 ml tricontanol in 200 l water and (vi) 200 ml tricontanol in 2000 ml water on three cultivars of sunflower, viz. Morden, HA-234B and KBSH-1. Spray of water constituted control. Spraying of TIBA combined with NAA had highest head diameter, number of filled seeds, seed filling percentage, seed yield, test weight, seed density and volume weight. Cultivar KBSH-1 produced maximum yield and yield components. Yield parameters, like test weight and seed density, differed significantly due to interaction of both growth regulators and cultivars.

Baydar and Gokmen (2003) performing an experiment on hybrid seed production in safflower, found that the spray of 100 ppm GA_3 on buds of less than 0.5 cm diameter of non-spiny variety (Dineer '5-118') and spiny variety ('5-154') at three successive growth stages (75, 82 and 89 DAS) did not affect the viability of achenes.

Khan *et al.* (2003) applied five pre-sowing treatment to seeds of four cultivars of sunflower (7-1A, 7-1B, RHA-271 and APSH-11). The seed treatments included (i) hydration for 24 h followed by the drying back to the original moisture level, (ii) cold hydration for 72 h at 10°C followed by the drying, (iii) hydration with 100 ppm GA_3 for 24 h followed by the drying, (iv) hydration for 24 h and the drying followed by dry dressing with thiram at the rate of 0.25% and (v) the untreated seed. They concluded that the hydration of seeds for 24 h followed by the drying proved best particularly for 7-1A and 7-1B.

Shivankar *et al.* (2003) studied the effect of pre-sowing seed treatment with potassium chloride, potassium dihdyrogen orthophosphate, manganese sulphate, potassium nitrate, thiourea, GA_3, Kn, hydration, hydration + thiram, thiram or *Trichoderma harzianum* on the performance of sunflower cv. Morden. They noted that the treatment with 50 ppm GA_3 increased seed yield significantly.

Siddiqui and Mohammad (2003) conducting an experiment on sunflower cv. Morden, studied the effect of pre-sowing seed treatment with four levels (10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}M) of four plant growth regulators, viz. GA_3, IAA, IBA and Kn, keeping water as the control, on nitrate reductase activity (NRA) and dry matter yield at 30, 60 and 90 DAS and on seeds per head, 1000-seed weight, seed yield per plant, oil content and oil yield per plant at harvest. The four plant growth regulators and their concentrations alone had a significant effect on NRA at 60 and 90 DAS, dry matter yield at 90 DAS and seeds per head, seed yield per plant and oil yield per plant at harvest. Among plant growth regulators, GA_3 proved to be the best at 10^{-5}M.

Paramarik and Basu (2004) studied the effect foliar spray of GA_3 and NAA on germination percentage, vigour index, root and shoot length and fresh and dry weight of four cultivars of safflower, namely A_1, A_{200}, A_{300} and Bhima. They applied GA_3 and NAA each at 50, 100 and 200 ppm. The mean germination percentage, vigour index and root and shoot length were higher with the application of NAA, whereas fresh and dry weight were higher with the application of GA_3. Cultivar A_{300} recorded the highest mean germination percentage, vigour index and root and shoot length, whereas cultivar A_1 gave the highest fresh and dry weight.

2.5 Concluding remarks

The above review of literature broadly establishes that plant growth hormones in general and GA_3 in particular have stimulative effect on growth and development of plants. Of the two methods of hormone application, pre-sowing seed treatment seems to be promising due to many factors, including the small amount of the hormone required and low operation cost involved. The review of literature also reveals that the duration for soaking treatments is not constant as various researches gave soaking treatments for different duration. The literature further imparts that comparatively less work has been done on sunflower. It is, therefore, highly desirable to extent the work by soaking the seeds of sunflower in aqueous solution of GA_3 of varying concentration for different duration.

Materials and Methods

CONTENTS

CHAPTER 3

MATERIALS AND METHODS

To achieve the objectives contrived in Chapter I, a factorial randomized design pot experiment was conducted on the locally poplar cultivar of *Helianthus annuus* L. (sunflower), namely PAC 3776 during 'zaid' (summer) season of 2008. The experiment was performed in a net house of the Department of Botany, the Aligarh Muslim University, Aligarh. The details of agro-climatic conditions, analyses of the homogenous mixture of soil and cowdung manure used for the filling of experimental pots and the techniques and procedures employed are given below.

3.1 Agro-climatic conditions

Aligarh is one of the seventy one districts of Uttar Pradesh (Anonymous, 2008). It is situated at 27°52'N latitude, 78°51'E longitude and 187.45 m altitude. It has semi-arid and sub-tropical climate, with severest hot dry summers and intense cold winters. The winter extends from the middle of October to the end of March. The mean temperatures for December and January, the coldest months, are about 15°C and 13°C respectively. The extreme minimum recorded for any single day is 2°C and 0.5°C respectively. The summer season extends form April to June and the average temperatures for May and June are 34.5°C and 34°C respectively. It sometime reaches up to 45°C for May and 45.5°C for June. The monsoon extends from the end of June to the middle of October and the mean temperature ranges between 26°C to 30°C. The mean annual rainfall is about 847.3mm. More than 85% of the total

down pour is delivered during a short span of four months from June to September. The remaining showers are received during winter.

The relative humidity of the winter season ranges between 56% to 77% with an average 66.5%. In the summer, it ranges between 37% to 49% with an average of 43%, whereas in the monsoon season, it ranges between 63% and 73% with an average of 68%.

Aligarh district has the same soil composition and appearances as those found generally in the plains of Uttar Pradesh. Different types of soil, such as sandy, loamy, sandy-loam and clayey-loam are found in the district.

3.2 Soil analysis

Just before sowing a composite soil sample, collecting from each pot, was analyzed for the soil characteristics. The soil sample was analyzed in the soil Testing Laboratory, Government Agriculture Farm, Quarsi, Aligarh. The physico-chemical properties of soil are given in Table1.

3.3 Filling of pots

Sixty four earthen pots of equal size (25 cm height and 25 cm diameter) were cleaned and filled in each with 4 kg homogenous mixture of soil and farmyard manure in the ratio of 4:1. These filled pots were arranged according to a factorial randomized design in the net house of the Department of Botany, A.M.U., Aligarh.

3.4 Seeds

Seeds of sunflower var. PAC 3776 were obtained from the Advanta India, Sikandarabad (A.P.) and their viability was tested before sowing. Seeds were surface sterilized by using 0.1% mercuric chloride solution (Appendix).

Table 1. Physico-chemical characteristics of the soil mixture used for the experiment

Texture	Sandy loam
pH (1:2)	7.6
Conductivity (1.2) dS/m	0.43
Available N (kg/ha)	190.45
Available P (kg/ha)	10.00
Available K (kg/ha)	209.00

Table 2. Scheme of treatments for the experiment (factorial randomized design)

Duration of soaking (h)	Concentration of GA_3 (M)			
	Water	10^{-7}	10^{-5}	10^{-3}
4				
8				
12				
16				

N.B. : A Uniform basal dose of 32 Kg N + 15 kg P + 17 Kg K kg/ha was applied.

3.5 Pot experiment

The experiment was performed according to a factorial randomized design during the 'zaid' (summer) season of 2008. The aim of the experiment was to study the effect of four concentrations and four soaking durations of pre-sowing seed treatment of GA_3 on the performance of sunflower CV. PAC 3776. Four concentrations of aqueous solution of GA_3, viz. water (0), 10^{-7}, 10^{-5} and 10^{-3} M GA_3, constituted one variant and four pre-sowing seed soaking durations (4, 8, 12 and 16 h), the other. A uniform recommended basal dose of 17.9 mg N + 11.6 mg P + 7.6 mg K/kg soil (40 kg N + 26 kg P + 17 kg K/ha) was applied to the soil. 10.5 mg N/kg soil (23.6 kg N/ha) and 11.6 mg P/kg soil

(26 kg P/ha) in the form of diammonium phosphate and 7.6 mg K/kg soil (17 kg K/ha) as muriate of potash were applied at the time of sowing. A total of 7.3 kg N/kg soil (16.4 kg N/ha) as urea was top-dressed in two equal splits, i.e. half 8.2 kg N/ha at 25 DAS and the remaining half at 50 DAS. Seeds from each treatment were sown at the rate of 10 seeds per pot. Thinning was performed after germination and four plants per pot were maintained.

The plants were kept free from weeds and irrigated as and when required. Each treatment was replicated four times. Scheme of treatments for the experiment is given in Table 2. Growth characters and physiological and biochemical parameters were recorded at 50 and 70 DAS and yield and quality characteristics at 90 DAS (harvest).

3.6 Sampling techniques

The performance of the crop was assessed on the basis of the following growth characters, physiological and biochemical parameters as also yield and oil quality characteristics :

3.6.1 Growth characters

The following growth characters were studied :

(i) Shoot length per plant

(ii) Root length per plant

(iii) Leaf area per plant

(iv) Leaf area index

(v) Shoot fresh weight per plant

(vi) Root fresh weight per plant

(vii) Shoot dry weight per plant

(viii) Root dry weight per plant

3.6.1.1 Determination of growth characters

3.6.1.1.1 Length of shoot and root

Length of shoot and root on per plant basis was determined separately with the help of a meter scale.

3.6.1.1.2 Leaf area per plant

Leaf area of a plant was obtained by gravimetric method. The leaf area of four leaves from each replicate was determined by tracing on graph sheets and dry weight for these leaves was recorded. The leaf area per plant was computed by using total leaf dry weight per plant and dry weight of those leaves for which the area was obtained. The following formula was used :

$$\text{Leaf area per plant} = \frac{LA_1 x W_2}{W_1}$$

LA_1 = leaf area of leaves traced on graph sheets

W_1 = Dry weight of leaves for which area was traced on graph sheets.

W_2 = Total leaf dry weight per plant

3.6.1.1.3 Leaf area index

LAI was determined by the following formula suggested by Watson (1958):

$$LAI = \frac{\text{Leaf area}}{\text{Ground area}}$$

3.6.1.1.4 Fresh weight of shoot and root

Weight of fresh matter of shoot and root was determined separately with the help of an electronic balance.

3.6.1.1.5 Dry weight of shoot and root

The shoot and root of each plant from each treatment were dried in a hot air over at 80°C for 24 h and the dry weight was obtained with the help of an electronic balance.

3.6.2 Physiological and biochemical parameters

The following physiological and biochemical parameters were studied in leaves :

(i) Chlorophyll content

(ii) Carbonic anhydrase activity

(iii) Nitrate reductase activity

(iv) Nitrogen content

(v) Phosphorus content

(vi) Potassium content

3.6.2.1 Determination of physiological and biochemical parameters

3.6.2.1.1 Chlorophyll content

Chlorophyll content was estimated following the method of Arnon (1949). The details are described below.

1 g finely cut fresh leaves were ground in 2 ml 80% acetone (Appendix) using a mortar and pestle. The acetone extract was allowed to centrifuge at 5,000 rpm for 5 min. the supernatant was collected into 100 ml volumetric flask. The residue was washed three times, with each washing performing with 5 ml 80% acetone. The washings were collected into the same volumetric flask and the volume was made up to the mark using 80% acetone. The optical

density (OD) was read at 645 and 663 nm against the blank (80% acetone) on a sepectrophotometer (Elico, India).

The chlorophyll content present in the extract was calculated using the following equations:

$$\text{Chlorophyll 'a' content} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W} \text{ mg/kg leaf tissue}$$

$$\text{Chlorophyll 'b' content} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W} \text{ mg/kg leaf tissue}$$

$$\text{Total chlorophyll content} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W} \text{ mg/kg leaf tissue}$$

Where,

A = Absorbance at the specific wavelengths

B = Final volume of chlorophyll extract in 80% acetone

W = Fresh mass of tissue used for extraction

3.6.2.1.2 Carbonic anhydrase activity

Carbonic anhydrase (CA) enzyme catalyses the reversible hydration of carbon dioxide (CO_2) to give the bicarbonate ion (HCO_3^-).

$$HO_2 + CO_2 \xrightarrow{CA} H^+ + HCO_3^-$$

CA activity in fresh leaves was estimated using the method described by Dwivedi and Randhawa (1974).

The fresh leaf samples were cut into small pieces at temperature below 25°C. 200 mg these pieces were weighed and transferred into Petri plates. The leaf pieces were taken in 10 ml of 0.2M cystein hydrochloride (Appendix) and

left for 20 min. at 4°C. The leaf pieces were blotted and transferred into a test tube containing 4 ml phosphate buffer of pH 6.8 (Appendix). Into the test tube, 4 ml 0.2M sodium bicarbonate ($NaHCO_3$) solution (Appendix) and 0.2 ml 0.002% bromothymol blue indicator (Appendix) were added. The test tube was shaken gently and left for 20 min. at 4°C. CO_2 liberated by the catalytic action of CA on $NaHCO_3$ was estimated by titrating the reaction mixture against 0.05 N hydrochloric (HCl) acid (Appendix) using methyl red (Appendix) as an indicator. In each sample, the quantity of HCl used to neutralize reaction mixture was noted and difference was calculated. A blank consisting of all the above components of reaction mixture, except the leaf sample, was run simultaneously with each set of sample. The activity of CA was expressed as molCO_2/kg (leaf FW)/s.

3.6.2.1.3 Nitrate reductase activity

The enzyme NR catalyses the reduction of nitrate (NO_3^-) to nitrite (NO_2^-).

$$NO_3^- + NADH + H^+ \xrightarrow{NR} NO_2^- + NAD + H_2O$$

The activity of NR in fresh leaves was measured following the method laid down by Jaworski (1971). The leaves were cut into small pieces (1 cm^2). 200 mg these chopped leaves were weighed and transferred into plastic vials. Into each vial, 2.5 ml phosphate buffer of pH 7.5 (Appendix) and 0.5 ml potassium nitrate solution (Appendix) were added followed by the addition of 2.5 ml 5% isopropanol (Appendix). These vials were incubated in a BOD incubator for 2h at 30+2°C in the dark. 0.4 ml incubated mixture was taken into a test tube into which 0.3 ml each of sulphanilamide solution and NED-HCl (Appendix) were added. The test tubes were left for 20 min. for maximum colour development.

The mixture was diluted to 5 ml by adding double distilled water (DDW). OD was recorded at 540 nm using the spectrophotometer. A bank consisting of all the above components, except the leaf sample, was run simultaneously with each set of determination.

3.6.2.1.3.1 Standard curve

30 mg sodium nitrite was dissolved in a sufficient amount of DDW and the final volume was made up to 1000 ml using DDW. From this solution, 0.2, 0.4, 0.6 .....2.0 ml were taken into separate test tubes. Into each of these, 0.3 ml each of 1% sulphanilamide and 0.02% NED-HCl was added. The solution was left for 20 min. for maximum colour development. The solution was diluted to 5 ml with DDW and OD was read at 540 nm on the spectrophotometer using a blank. A standard curve was plotted using the concentration of sodium nitrite solution versus OD.

The sample reading was compared with the standard curve and NRA was expressed as n mol NO_2/g (leaf fresh weight)/h

3.6.2.1.4 Nitrogen content

For estimation of N, P and K, the leaf powder was digested by the method of Lindner (1944) given below.

100 mg dried powder was taken into a 50 ml Kjeldahl flask. Into this, 2 ml concentrated sulphuric acid was added and the mixture was heated for 2 h. After cooling for about 15 min., 0.5 ml chemically pure 30% hydrogen peroxide (H_2O_2) was added drop by drop to the black coloured mixture and the contents of the flask were heated till the colour turned from black to light yellow. This procedure was repeated till a clear solution is obtained. The

peroxide digested material was transferred into a 100 ml volumetric flask followed by three washings each with 5 ml DDW and the volume was made up to the mark.

Leaf N content was estimated following the method of Lindner (1944).A 10 ml of the peroxide digested material was taken into a 50 ml volumetric flask into which 2 ml 2.5 N sodium hydroxide (Appendix) and 1 ml 10% sodium silicate solution were added to neutralize excess of acid and to prevent turbidity, respectively. The volume of the solution was made up to the mark with the help of DDW. Into a 10 ml graduated test tube, 5 ml of this solution was taken and 0.5 ml Nessler's reagent was added. The final volume was made 10 ml with DDW. After waiting for 5 min. to develop the colour, OD of the solution was determined at 525 nm on the spectrophotometer using a blank. The blank was consisted of all the above components except DDW in place of the peroxide digested material.

3.6.2.1.4.1 Standard curve

A standard solution was prepared by dissolving 25 mg ammonium sulphate in the sufficient amount of DDW and the final volume was made 500 ml with DDW. From this solution, 0.1, 0.2, 0.3 ... 1.0 ml were taken into 10 test tubes separately. Into each test tube, dilution was made up to 5 ml with DDW followed by addition of 0.5 ml Nessler's reagent. A yellow colour of varied intensities developed in each test tube. The OD of each intensity was read at 525 nm on the spectrophotometer using the blank. A calibration curve was plotted using the concentration of ammonium sulphate solution versus OD.

The sample reading was compared with the standard curve and N content was expressed as percentage of dry weight.

3.6.2.1.5 Phosphorus content

Leaf P content in the peroxide digested material was estimated by the method of Fiske and Subba Row (1925). 5 ml digested material of each sample was taken into a 10 ml graduated test tube into which 1 ml molybdic acid (Appendix) was added carefully followed by the addition 0.4 ml 1-amino-2-naphthol 4-sulphonic acid (Appendix). The colour turned blue. The solution was made up to 10 ml with the help of DDW. The solution was shaken for 5 min. and then transferred into a spectrophotometer tube and OD was read at 620 nm on the spectrophotometer using a blank consisted of all the above components except DDW in place of the peroxide material.

3.6.2.1.5.1 Standard curve

350 mg potassium dihdyrogen orthophosphate (KH_2PO_4) was dissolved in the sufficient amount of DDW followed by the addition of 10 ml 10 N sulphuric acid (Appendix), with the final volume being 1000 ml with DDW. From this stock solution, 0.1, 0.2, 0.3,... 1.0 ml concentration were taken into 10 different test tubes. Into each test tube, 1` ml molybdic acid and 0.4 ml of 1-amino -2naphthol 4-sulphonic acid were added. After 5 min., OD was read at 625 nm on the spectrophotometer. A standard curve was prepared using different dilutions of KH_2PO_4 solution versus OD.

The OD of the sample was compared with the standard curve and P content was expressed in terms of percentage of dry weight.

3.6.2.1.6 Potassium content

Leaf K content was estimated flame photometrically. A 10 ml of the peroxide digested material was taken to read potassium content on a flame

photometer (Fotoflame, AIMIL). A blank (DDW) was also run with each set of determination.

3.6.2.1.6.1 Standard curve

1.91g potassium chloride (KCl) was dissolved in the sufficient amount of DDW, with the final volume being 1000ml with DDW. The resulting solution was 10 ppm K. From this solution 1, 2, 3... and 10 ml solution transferred into different plastic vials. After diluting it to 10 ml with DDW in each vial, the solution was run into the photometer using the blank. A calibration curve was plotted using known dilutions of KCl versus reading on the photometer.

The reading of the sample was compared with the standard curve and K content was expressed as percentage of dry weight.

3.6.3 Yield characteristics

The following yield characteristics were studied:

(i) Head diameter

(ii) Seeds per head

(iii) 1000-seed weight

(iv) Seed yield per plant

(v) Biological yield per plant

(vi) Harvest index

(vii) Oil content

(viii) Oil yield per plant

3.6.3.1 Determination of yield characteristics

3.6.3.1.1 Head diameter

The diameter of head of plant samples was measured with the help of a meter scale.

3.6.3.1.2 Seeds per head

The number of seeds per head was determined by counting the number of seeds of four heads.

3.6.3.1.3 1000-seed weight

The weight of 1000 seeds was determined with the help of an electronic balance.

3.6.3.1.4 Seed yield per plant

The total seeds of four plants (four heads) were threshed, cleaned and allowed to dry in the sun for some time and their weight was obtained with the help of an electronic balance, with expressing their weight on per plant basis.

3.6.3.1.5 Biological yield per plant

The biological yield was recorded before the threshing of plants. It was determined by weighing the dry mass of four complete plants with the help of an electronic balance, with expressing the yield on per plant basis.

3.6.3.1.6 Harvest index

The harvest index was computed by dividing the seed yield (economic yield) of a plant by the biological yield of the plant and expressed on per cent basis.

$$\text{Harvest index} = \frac{\text{Seed yield}}{\text{Biological yield}} \times 100$$

3.6.3.1.7 Oil content

10 g ground seed meal was transferred into a Soxhlet apparatus and the sufficient quantity of petroleum ether was added. The apparatus was kept on a hot water bath running at 60°C for about 6 h for complete extraction of the oil. The oil content was expressed as a percentage of seeds and was calculated by the following formula:

$$\text{Oil content (\%)} = \frac{m_o}{m_s} \times 100$$

Where,

m_o = mass of the extracted oil

m_s = mass of seed sample used

3.6.3.1.8 Oil yield

The oil yield per plant was computed on the basis of the oil percentage and seed yield per plant.

3.6.4 Oil quality characteristics

The following characteristics of oil were studied:

(i) Acid value

(ii) Iodine value

(iii) Saponification value

3.6.4.1 Determination of oil quality characteristics

3.6.4.1.1 Acid value

The acid value of oil is the number of mg of potassium hydroxide (KOH) used to neutralize free acids in one gram of oil (mg KOH/g oil). It was determined by the titration method described below (Anonymous, 1970).

2 g oil was dissolved in 50 ml solvent mixture of 95% ethyl alcohol and diethyl ether (1:1) in a 250 ml conical flask. Titration was carried out with 0.1N KOH (Appendix) using phenolphthalein (Appendix) as an indicator and the amount of ml 'a' of 0.1N KOH required was noted. The acid value was determined by the following formula.

$$\text{Acid value} = \frac{\text{'a'} \times 0.00561 \times 1000}{W}$$

where,

'a' = ml of 0.1 N KOH used in titration

W = weight of oil in g

3.6.4.1.2 Iodine value

The iodine value of an oil is the number of g of iodine absorbed by 100 g oil (g I/100 g oil). It was determined by using iodine monochloride method describe below (Anonymous, 1970).

2 g oil was placed into a dry 250ml round bottom flask. 10 ml carbon tetrachloride and 20 ml iodine monochloride solution (Appendix) were add. The flask was stoppered and allowed to stand in a dark place for about 30 min. There after, 15 ml potassium iodide solution (Appendix) and 100 ml DDW

were poured into the flask with gentle shaking. Titration was carried out with 0.1 N sodium thiosulphate solution (Appendix), using starch solution (Appendix) as an indicator. The number of ml 'a' of sodium thiosulphate used was noted. For blank, similar operation was performed but without the oil, and the number of ml 'b' of 0.1 N sodium thiosulphate solution used was noted. Iodine value was calculated by the following formula:

$$\text{Iodine value} = \frac{(\text{'b'} - \text{'a'}) \times 0.01269 \times 100}{W}$$

Where,

'a' = number of ml of 0.1 N sodium thiosulphate solution used for the sample

'b' = number of ml of 0.1 N sodium thiosulphate solution used for the blank

W = weight of oil in g

3.6.4.1.3 Saponification value

The saponification value of oil is the number of mg of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of oil (mg KOH/g oil). It was determined by titration method described below (Anonymous 1970).

2 g oil was taken into a 250 ml conical flask to which 25 ml 0.5 N KOH solution (Appendix) was added. The flask was attached with a reflux condenser and heated on a water bath for about 1 h. with frequent rotation of the contents of the flask. After cooling, 1 ml phenolphthalein solution was added. The excess of alkali was titrated with 0.5 N hydrochloric acid (Appendix) and the

number of ml 'a' was noted. For blank, the operation was repeated in the same manner omitting the oil, and the number of ml 'b' required was noted. Saponification value was calculated by following formula:

$$\text{Saponification value} = \frac{(\text{'b'} - \text{'a'}) \times 0.02805 \times 1000}{W}$$

where,

'a' = number of ml of 0.5 N HCl used for the sample

'b' = number of ml of 0.5 N HCl used for the blank

w = weight of oil in g

3.7 Statistical analysis

The data of the experiment were analyzed statistically by adopting the analysis of variance technique according to Gomez and Gomez (1984). For the 'F' test, the error due to replicates was also determined. When 'F' value was found to be significant at 5% level of probability, critical difference (CD) was calculated. The model of analysis of variance for the design employed is given in Table 3.

Table 3. Model of analysis of variance (ANOVA) of the experiment. (Factorial randomized design)

Source of variation	DF	SS	MSS	F
Replications	3			
Soaking treatments (C)	3			
Soaking durations(D)	3			
CXD	9			
Error	45			
Total	63			

Experimental Results

CONTENTS

CHAPTER 4

EXPERIMENTAL RESULTS

In a pot experiment, effect of four concentrations and four soaking durations of pre-sowing seed treatment of GA_3 was studied on the performance of sunflower. The data for various parameters studied are summarized below.

4.1 Growth characters

The effect of GA_3 soaking concentrations and durations alone or in combination was significant on growth parameters at 50 and 70 DAS, except concentration effect on root dry weight at 70 DAS, soaking duration effect on leaf area at 50 DAS and shoot fresh and dry weight at 70 DAS and interaction effect on shoot fresh weight and root dry weight at 70 DAS (Tables 4-11).

4.1.1 Shoot length per plant

Soaking treatment 10^{-5}M GA_3 gave the maximum plant height at both 50 and 70 DAS. Its effect was followed by that of 10^{-3}M GA_3 at 50 DAS and by 10^{-3}M GA_3, 10^{-7}M GA_3 and 0 M GA_3 at 70 DAS. Soaking with 10^{-5}M GA_3 gave 13.12% and 3.21% taller plants than 0 M GA_3 at 50 and 70 DAS respectively.

Soaking for 8 h proved best at both stages. However, its effect was at par with that of 12 h soaking. Soaking for 8 h gave 3.28% and 1.06% higher shoot length than 4 h soaking at 50 and 70 DAS respectively.

Interaction 10^{5}M GA_3 x 8 h gave the maximum height at both stages. However, its effect was at par with that of 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h and 10^{-5}M GA_3 x 4 h at both stages and also by 10^{-3}M GA_3 x 8 h at 50

DAS. Interaction 10^{-5}M GA_3 x 8 h gave 16.67% and 4.48% higher shoot length than the lowest value giving interaction 0 M GA_3 x 4 h at 50 and 70 DAS respectively (Table 4).

4.1.2 Root length per plant

Soaking treatment 10^{-5}M GA_3 gave the maximum root length at both 50 and 70 DAS. Its effects was followed by that of 10^{-3}M GA_3 at both stages and also by 10^{-7}M GA_3 at 70 DAS. Soaking with 10^{-5}M GA_3 gave 84.69% and 15.68% higher value than 0 M GA_3 at 50 and 70 DAS respectively.

Soaking for 8 h proved best at the both stages. Its effect was followed at 50 DAS but equalled at 70 DAS by that of 12 h soaking. Soaking for 8 h gave 10.87% and 3.06% higher root length than 4 h soaking at 50 and 70 DAS respectively.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum root length at both stages, however its effect was at par with that of 10^{-5}M GA_3 x 12 h. Interaction 10^{-5}M GA_3 x 8 h gave 108.89% and 19.78% higher root length than the lowest value giving interaction 0 M GA_4 x 4 h at 50 and 70 DAS respectively (Table 5).

4.1.3 Leaf area per plant

Soaking treatment 10^{-5}M GA_3 gave the maximum leaf area at both 50 and 70 DAS. Its effect was at par with that of 10^{-3}M GA_3. Soaking with 10^{-5}M GA_3 gave 19.48% and 49.60% higher leaf area than 0 M GA_3 at 50 and 70 DAS respectively.

The effect of soaking durations was not found significant on this parameter at 50 DAS. At 70 DAS, soaking for 8 h proved best, with its effect

Table 4. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on shoot length per plant (cm) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	36.00	38.00	41.00	39.80	38.70
8	37.50	39.60	42.00	40.80	39.97
12	37.00	39.20	41.80	40.60	39.65
16	36.50	39.00	41.50	40.00	39.25
Mean	36.75	38.95	41.57	40.30	
CD at 5%	C=0.61	D=0.61		C×D=1.21	
			70 DAS		
4	64.00	65.15	66.20	65.25	65.15
8	64.85	65.70	66.87	65.97	65.84
12	64.80	65.50	66.60	65.75	65.66
16	64.25	65.35	66.50	65.60	65.42
Mean	64.47	65.42	66.54	65.64	
CD at 5%	C=0.36	D=0.36		C×D=0.72	

Table 5. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on root length per plant (cm) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	4.50	6.50	8.70	7.90	6.90
8	5.30	7.40	9.40	8.50	7.65
12	5.00	7.00	9.20	8.20	7.35
16	4.80	6.80	8.90	8.00	7.13
Mean	4.90	6.92	9.05	8.15	
CD at 5%	C=0.24	D=0.24		C×D=0.47	
			70 DAS		
4	13.40	14.05	15.55	14.50	14.37
8	13.70	14.65	16.05	14.85	14.81
12	13.65	14.55	15.85	14.60	14.66
16	13.58	14.20	15.40	14.35	14.38
Mean	13.58	14.36	15.71	14.58	
CD at 5%	C=0.24	D=0.24		C×D=0.47	

being followed by that of 12 h soaking. Soaking for 8 h gave 13.68% higher leaf area than 4 h soaking at this stage.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value at both stages. However, its effect was at par with that of 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h, 10^{-5}M GA_3 x 4 h, 10^{-3}M GA_3 x 8 h and 10^{-3}M GA_3 x 12 h at 50 DAS and was followed by that of 10^{-5}M GA_3 x 12 h at 70 DAS. Interaction 10^{-5}M GA_3 x 8 h gave 21.09% and 77.10% more leaf area than the lowest value giving interaction 0 M GA_3 x 4 h at 50 and 70 DAS respectively (Table 6).

4.1.4 Leaf area index

Soaking treatment 10^{-5}M GA_3 gave the maximum LAI at both 50 and 70 DAS. However, its effect was at par with that of 10^{-3}M GA_3 and 10^{-7}M GA_3 at 50 DAS and only with that of 10^{-3}M GA_3 at 70 DAS. Soaking with 10^{-5}M GA_3 gave 9.46% and 11.14% higher LAI than 0 M GA_3 at 50 and 70 DAS respectively.

Soaking for 8 h proved best at both stages. Its effect was followed at 50 DAS but equalled at 70 DAS by that of 12 h soaking. Soaking for 8 h gave 19.81% and 11.35% higher LAI than 4 h soaking at 50 and 70 respectively.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum LAI at both 50 and 70 DAS. However, its effect was at par with that of 10^{-3}M GA_3 x 8 h, 10^{-7}M GA_3 x 8 h, 10^{-5}M GA_3 x 12 h at 50 DAS and also by 10^{-5}M GA_3 x 12 h, 10^{-3}M GA_3 x 12 h and 10^{-7}M GA_3 x 12 h at 70 DAS. Interaction 10^{-5} GA_3 x 8 h gave 33.82% and 22.94% higher leaf area index than the lowest value giving interaction 0 M GA_3 x 4 h at 50 and 70 DAS respectively (Table 7).

Table 6. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on leaf area per plant (cm^2) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	190.50	210.25	230.37	220.25	212.84
8	200.00	210.87	230.68	220.75	215.58
12	190.75	210.75	230.62	220.50	213.16
16	190.62	210.37	230.50	220.43	212.98
Mean	192.96	210.56	230.54	220.48	
CD at 5%	C=0.61	D=NS		CxD=1.21	
			70 DAS		
4	260.13	340.06	380.06	368.04	337.07
8	310.87	380.43	460.69	380.68	383.17
12	310.06	360.37	450.06	370.87	372.84
16	270.81	350.31	432.35	360.81	353.57
Mean	287.96	357.79	430.79	370.10	
CD at 5%	C=3.57	D=3.57		CxD=7.13	

NS = Non-significant

Table 7. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on leaf area index of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	2.04	2.07	2.24	2.13	2.12
8	2.30	2.52	2.73	2.62	2.54
12	2.27	2.30	2.49	2.37	2.35
16	2.25	2.26	2.25	2.15	2.23
Mean	2.22	2.28	2.43	2.32	
CD at 5%	C=0.18	D=0.18		C×D=0.35	
			70 DAS		
4	4.01	4.26	4.32	4.36	4.23
8	4.36	4.72	4.93	4.84	4.71
12	4.30	4.68	4.76	4.70	4.61
16	4.21	4.45	4.75	4.58	4.49
Mean	4.22	4.52	4.69	4.62	
CD at 5%	C=0.14	D=0.14		C×D=0.27	

Table 8. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on shoot fresh weight per plant (g) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	5.07	7.15	9.02	8.46	7.42
8	5.13	7.25	9.28	8.85	7.62
12	5.10	7.22	9.24	8.81	7.59
16	5.09	7.19	9.15	8.78	7.55
Mean	5.09	7.20	9.17	8.73	
CD at 5%	C=0.27	D=NS		C×D=0.53	
			70 DAS		
4	19.43	20.68	21.15	21.08	20.59
8	19.64	20.98	21.24	21.12	20.75
12	19.62	20.71	21.18	21.10	20.66
16	19.55	20.69	21.17	21.09	20.63
Mean	19.56	20.77	21.19	21.10	
CD at 5%	C=0.93	D=NS		C×D=NS	

NS = Non-significant

Interaction 10^{-5}M GA_3 x 8 h gave the maximum root fresh weight at 50 and 70 DAS. However, its effect was at par with that of 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h, 10^{-5}M GA_3 x 4 h and 10^{-3}M GA_3 x 8 h at both stages. Interaction 10^{-5}M GA_3 x 8 h gave 64.88% and 37.09% higher root fresh weight than the lowest value giving interaction 0 M GA_3 x 4 h at 50 and 70 DAS respectively (Table 9).

4.1.7 Shoot dry weight per plant

Soaking treatment 10^{-5}M GA_3, followed by 10^{-3}M GA_3, gave the maximum value at both stages. Soaking with 10^{-5}M GA_3 gave 125.0% and 52.38% higher value than 0 M GA_3 at 50 and 70 DAS respectively.

The effect of soaking durations on shoot dry weight was not found significant at both stages.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value at both stages. However, its effect was at par with that of 10^{-5}M GA_3 x 12 h, 10^{-4}M GA_3 x 16 h and 10^{-5}M GA_3 x 4 h at both stages and also with that of 10^{-3}M GA_3 x 8 h at 70 DAS. Interaction 10^{-5}M GA_3 x 8 h gave 132.98% and 53.99% higher shoot dry weight than the lowest value giving interaction 0 M GA_3 x 4 h at 50 and 70 DAS respectively (Table 10).

4.1.8 Root dry weight per plant

Soaking treatment 10^{-5}M GA_3 gave the maximum value for root dry weight at 50 DAS, with its effect being followed by that of 10^{-3}M GA_3. Soaking with 10^{-3}M GA_3 gave 150.41% higher value than 0 M GA_3 at this stage. However, soaking concentrations did not vary in their effect at 70 DAS.

Table 9. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on root fresh weight per plant (g) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	0.598	0.712	0.932	0.845	0.771
8	0.635	0.789	0.986	0.899	0.827
12	0.624	0.764	0.972	0.882	0.810
16	0.604	0.742	0.960	0.863	0.792
Mean	0.615	0.751	0.962	0.872	
CD at 5%	C=0.05	D=0.05		CxD=0.09	
			70 DAS		
4	4.61	5.56	6.22	6.11	5.63
8	4.76	5.97	6.32	6.24	5.82
12	4.69	5.85	6.31	6.17	5.75
16	4.65	5.76	6.29	6.13	5.71
Mean	4.68	5.78	6.29	6.16	
CD at 5%	C=0.07	D=0.07		CxD=0.13	

Table 10. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on shoot dry weight per plant (g) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	0.94	1.01	2.13	1.85	1.48
8	0.98	1.13	2.19	1.88	1.55
12	0.97	1.06	2.17	1.87	1.52
16	0.96	1.02	2.15	1.86	1.51
Mean	0.96	1.05	2.16	1.86	
CD at 5%	C=0.09	D=NS		CxD=0.17	
			70 DAS		
4	3.13	3.83	4.78	4.29	4.01
8	3.17	3.91	4.82	4.41	4.08
12	3.17	3.89	4.81	4.35	4.06
16	3.14	3.85	4.79	4.33	4.03
Mean	3.15	3.87	4.80	4.34	
CD at 5%	C=0.23	D=NS		CxD=0.46	

NS = Non-significant

Soaking for 8 h proved best at 50 DAS, with its effect being at par with that of 12 h soaking. Soaking for 8 h gave 12.81% higher root dry weight than 4 h soaking at this stage. The effect soaking durations was not found significant on this parameter at 70 DAS.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum root dry weight at 50 DAS, with its effect being at par with that of 10^{-5}M GA_3 x 12 h and 10^{-5}M GA_3 x 16 h. Interaction 10^{-5}M GA_3 x 8 h gave 170.59% higher root dry weight than the lowest value giving interaction 0 M GA_3 x 4 h at this stage. However, interaction effect on this parameter was not found significant at 70 DAS (Table 11).

4.2 Physiological and biochemical parameters

Effect of GA_3 soaking concentrations and durations alone or in combination was significant on physiological and biochemical parameters at both stages, except concentration as well as duration effect on chlorophyll content and leaf P content at 50 DAS and interaction effect on chlorophyll content, CA activity as also leaf P content at 50 DAS and leaf N content at both stage (Tables 12-17).

4.2.1 Total chlorophyll content

Soaking treatment of 10^{-5}M GA_3 gave the maximum value for total chlorophyll content at 70 DAS, however its effect was at par with that of 10^{-3}M GA_3. Soaking with 10^{-5}M GA_3 gave 3.59% higher value than 0 M GA_3 at 70 DAS. However, soaking treatments proved equally effective at 50 DAS.

Soaking for 8 h proved the best at 70 DAS, however its effect was at par with that of 12 h soaking. Soaking for 8 h gave 4.19% higher total chlorophyll

Table 11. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on root dry weight per plant (g) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	0.119	0.140	0.295	0.260	0.203
8	0.130	0.182	0.322	0.282	0.229
12	0.125	0.158	0.314	0.274	0.217
16	0.121	0.145	0.304	0.263	0.208
Mean	0.123	0.156	0.308	0.269	
CD at 5%	C=0.013	D=0.013		C×D=0.208	
			70 DAS		
4	1.14	1.16	1.19	1.17	1.16
8	1.17	1.19	1.24	1.19	1.17
12	1.16	1.18	1.21	1.18	1.22
16	1.15	1.16	1.21	1.18	1.18
Mean	1.17	1.20	1.18	1.18	
CD at 5%	C=NS	D=NS		C×D=NS	

NS = Non-significant

content than 4 h soaking at 70 DAS. However, a non-significant effect was noted at 50 DAS.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value at 70 DAS. However, its effect was at par with that of 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h, 10^{-5}M GA_3 x 4 h, 10^{-3}M GA_3 x 8 h, 10^{-3}M GA_3 x 12 h, 10^{-7}M GA_3 x 8 h and 0 M GA_3 x 8 h at 70 DAS. Interaction 10^{-5}M GA_3 x 8 h gave 7.32% higher value than the lowest value giving interaction 0 M GA_3 x 4 h at 70 DAS. However, interactions did not affect at 50 DAS (Table 12).

4.2.2 Carbonic anhydrase activity

Soaking treatment of 10^{-5}M GA_3 gave the maximum value at both stages. Its effect was at par at 50 DAS and followed at 70 DAS by that of 10^{-3}M GA_3. Soaking with 10^{-5}M GA_3 gave 16.07% and 11.07% higher value than 0 M GA_3 at 50 and 70 DAS respectively.

Soaking for 8 h proved the best at both stages, however its effect was at par with that of 12 h soaking. Soaking for 8 h gave 21.68% and 33.71% higher value than 4 h soaking at 50 and 70 DAS respectively.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value for CA activity at 70 DAS, however its effect was at par with that of 10^{-3}M GA_3 x 8 h. Interaction 10^{-5}M GA_3 x 8 h gave 44.88% higher value than the lowest value giving interaction 0 M GA3 x 4 h at 70 DAS. Effect of interactions on this parameter was, however not significant at 50 DAS (Table 13).

4.2.3 Nitrate reductase activity

Soaking treatment of 10^{-5}M GA_3 gave the maximum value at both stages, with its effect being followed by that of 10^{-3}M GA_3. Soaking with

Table 12. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on total chlorophyll content (mg/g F W) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	1.39	1.40	1.42	1.41	1.40
8	1.47	1.50	1.52	1.51	1.50
12	1.44	1.45	1.47	1.46	1.45
16	1.41	1.43	1.45	1.44	1.43
Mean	1.42	1.44	1.46	1.45	
CD at 5%	C=NS	D=NS		C×D=NS	
			70 DAS		
4	1.64	1.66	1.71	1.70	1.67
8	1.72	1.73	1.76	1.75	1.69
12	1.69	1.70	1.74	1.72	1.73
16	1.66	1.68	1.72	1.69	1.71
Mean	1.67	1.74	1.71	1.68	
CD at 5%	C=0.03	D=0.03		C×D=0.05	

NS = Non-significant

Table 13. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on carbonic anhydrase activity [mol CO_2/kg (leaf F W)/s) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	2.07	2.28	2.36	2.34	2.26
8	2.56	2.68	2.92	2.85	2.75
12	2.18	2.53	2.65	2.58	2.49
16	2.13	2.35	2.47	2.41	2.34
Mean	2.24	2.46	2.60	2.55	
CD at 5%	C=0.069	D=0.069		C×D=NS	
			70 DAS		
4	2.54	2.62	2.75	2.65	2.64
8	3.32	3.48	3.68	3.64	3.53
12	3.27	3.34	3.51	3.40	3.38
16	2.80	2.92	3.28	3.14	3.04
Mean	2.98	3.09	3.31	3.21	
CD at 5%	C=0.057	D=0.057		C×D=0.115	

NS = Non-significant

10^{-5}M GA_3 gave 11.68% and 16.70% higher value than 0 M GA_3 at 50 and 70 DAS respectively.

Soaking for 8 h proved the best at both stages, with its effect being followed by that of 12 h soaking. Soaking for 8 h gave 11.59% and 12.02% higher value than 4 h soaking at 50 and 70 DAS respectively.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value at both stages. Its effect was followed by that of 10^{-3}M GA_3 x 8 h, 10^{-5}M GA_3 x 12 h, 10^{-7}M GA_3 x 8 h and 10^{-5}M GA_3 x 16 h at 50 DAS. However, effect of 10^{-5}M GA_3 x 8 h was at par with that of 10^{-3}M GA_3 x 8 h at 70 DAS. Interaction 10^{-5}M GA_3 x 8 h gave 25.86% and 28.78% higher value than the lowest value giving interaction 0 M GA_3 x 4 h at 50 and 70 DAS respectively (Table 14).

4.2.4 Nitrogen content

Soaking treatment of 10^{-3}M GA_3 gave the maximum for leaf N content at both 50 and 70 DAS. Its effect was followed by 10^{-5}M GA_3 at both stages. Soaking with 10^{-3}M GA_3 gave 5.67% and 12.80% higher value than 0 M GA_3 at 50 and 70 DAS respectively.

Soaking for 8 h proved the best at both stages, however its effect was followed by that of 12 h soaking. Soaking for 8 h gave 13.98% and 10.76% higher value than 4 h soaking at 50 and 70 DAS respectively.

Effect of interactions on this parameter was not found significant at both 50 and 70 DAS (Table 15).

4.2.5 Phosphorus content

Soaking treatment of 10^{-3}M GA_3 gave the maximum value for leaf P content at 70 DAS. Its effect was followed by that of 10^{-5}M GA_3 at this stage.

Table 14. **Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on nitrate reductase activity (n mol NO_2^-/g /(leaf F W)/h) of sunflower at 50 and 70 DAS (Mean of four replicates)**

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	245.34	255.91	272.83	266.49	260.14
8	274.84	285.53	308.79	293.98	290.29
12	260.15	268.61	287.64	281.30	274.43
16	253.80	264.37	283.41	270.72	268.08
Mean	258.03	268.60	288.17	278.12	
CD at 5%	C=5.71	D=5.71		CxD=11.41	
			70 DAS		
4	293.98	315.14	348.98	325.72	320.96
8	336.28	355.33	378.59	368.02	359.55
12	310.91	329.94	363.79	346.86	337.88
16	300.33	321.48	357.44	338.12	327.09
Mean	310.38	330.47	362.20	344.68	
CD at 5%	C=6.64	D=6.64		CxD=13.27	

Table 15. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on leaf nitrogen content (%) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	2.28	2.40	2.57	2.63	2.47
8	2.47	2.53	2.68	2.75	2.61
12	2.36	2.49	2.64	2.72	2.55
16	2.32	2.44	2.59	2.66	2.50
Mean	2.36	2.47	2.62	2.69	
CD at 5%	C=0.040	D=0.040		CxD=NS	
			70 DAS		
4	2.37	2.44	2.57	2.65	2.51
8	2.60	2.74	2.81	2.96	2.78
12	2.57	2.66	2.77	2.89	2.72
16	2.46	2.59	2.68	2.78	2.63
Mean	2.50	2.61	2.71	2.82	
CD at 5%	C=0.049	D=0.049		CxD=NS	

NS = Non-significant

Soaking with 10^{-3}M GA_3 gave 43.30% higher value than 0 M GA_3 at 70 DAS. However, a non-significant effect of soaking treatments was noted at 50 DAS.

Soaking for 8 h proved the best at 70 DAS. Its effect was followed by that of 12 h soaking at this stage. Soaking for 8 h gave 24.23% higher value than 4 h soaking at 70 DAS. Duration treatments did not affect on this parameter at 50 DAS.

Interaction 10^{-3}M GA_3 x 8 h gave the maximum value at 70 DAS, however its effect was at par with that of 10^{-5}M GA_3 x 8 h. Interaction 10^{-3}M GA_3 x 8 h gave 67.63% higher value than the lowest value giving interaction 0 M GA_3 x 4 h at 70 DAS. Interaction effect did not vary at 50 DAS (Table 16).

4.2.6 Potassium content

Soaking treatment of 10^{-3}M GA_3 gave the maximum for leaf K content at both 50 and 70 DAS. However, its effect was at par with that of 10^{-5}M GA_3 at both stages and also by 10^{-7}M GA_3 at 70 DAS. Soaking with 10^{-3}M GA_3 gave 32.48% and 32.79% higher value than 4 h soaking at 50 and 70 DAS respectively.

Soaking for 8 h proved the best at both stages, with its effect being followed by that of 12 h soaking. Soaking for 8 h gave 45.38% and 44.35% higher value than 4 h soaking at 50 and 70 DAS respectively.

Interaction 10^{-3}M GA_3 x 8 h gave the maximum value at both stages. However, its effect was at par with that of 10^{-5}M GA_3 x 8 h at both stages and also by 10^{-7}M GA_3 x 8 h at 70 DAS. Interaction 10^{-3}M GA_3 x 8 h gave 86.14% and 85.71% higher value than the lowest value giving interaction 0 M GA_3 x 4 h at 50 and 70 DAS respectively (Table 17).

Table 16. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on leaf phosphorus content (%) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	0.240	0.288	0.360	0.375	0.315
8	0.310	0.345	0.387	0.393	0.358
12	0.289	0.322	0.374	0.389	0.343
16	0.264	0.315	0.365	0.382	0.331
Mean	0.275	0.317	0.371	0.384	
CD at 5%	C=NS	D=NS		C×D=NS	
			70 DAS		
4	0.312	0.342	0.420	0.488	0.388
8	0.402	0.487	0.514	0.523	0.482
12	0.353	0.460	0.496	0.507	0.454
16	0.340	0.448	0.476	0.492	0.439
Mean	0.351	0.434	0.477	0.503	
CD at 5%	C=0.006	D=0.006		C×D=0.013	

NS = Non-significant

Table 17. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on leaf potassium content (%) of sunflower at 50 and 70 DAS (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			50 DAS		
4	1.01	1.19	1.27	1.28	1.19
8	1.58	1.69	1.76	1.88	1.73
12	1.04	1.56	1.55	1.57	1.43
16	1.03	1.29	1.31	1.48	1.28
Mean	1.17	1.43	1.48	1.55	
CD at 5%	C=0.07	D=0.07		CxD=0.13	
			70 DAS		
4	1.05	1.26	1.32	1.34	1.24
8	1.63	1.76	1.83	1.95	1.79
12	1.12	1.61	1.62	1.64	1.50
16	1.07	1.35	1.36	1.56	1.34
Mean	1.22	1.49	1.53	1.62	
CD at 5%	C=0.14	D=0.14		CxD=0.27	

4.3 Yield characteristics

The effect of GA_3 soaking concentrations and durations alone or in combination was significant on all yield parameters studied at harvest, except duration effect on 1000-seed weight, seed yield per plant, harvest index and oil content and interaction effect on 1000-seed weight and biological yield per plant (Tables 18-20).

4.3.1 Head diameter

Soaking treatment 10^{-5}M GA_3 gave the maximum value for head diameter. Its effect was followed by that of 10^{-3}M GA_3. Soaking with 10^{-5}M GA_3 gave 47.02% higher value than 0 M GA_3.

Soaking for 8 h gave the maximum value at 90 DAS. However its effect was at par with that of 12 h soaking. Soaking for 8 h gave 11.74% higher value than 4 h soaking.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value for head diameter, however its effect was at par with that of 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h and 10^{-3}M GA_3 x 8 h. Interaction 10^{-5}M GA_3 x 8 h gave 70.0% higher value than the lowest value giving interaction 0 M GA_3 x 4 h (Table 18).

4.3.2 Seeds per head

Soaking treatment 10^{-5}M GA_3, followed by 10^{-5}M GA_3, gave the maximum value. Soaking with 10^{-5}M GA_3 gave 108.51% higher value than 0 M GA_3.

Soaking for 8 h proved the best. Its effect was followed by that of 12 h soaking. Soaking for 8 h gave 5.32% higher value than 4 h soaking.

Table 18. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on head diameter and seeds per head of sunflower at harvest (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			Head diameter (cm)		
4	5.0	6.8	7.7	7.4	6.73
8	6.0	7.6	8.5	8.0	7.52
12	5.7	7.4	8.3	7.8	7.30
16	5.4	6.9	8.0	7.6	6.98
Mean	5.53	7.18	8.13	7.70	
CD at 5%	C=0.32	D=0.32		C×D=0.63	
			Seeds per head		
4	315.0	462.0	680.0	534.0	497.75
8	350.0	492.0	704.0	551.0	524.25
12	339.0	483.0	696.0	543.0	515.25
16	324.0	470.0	689.0	538.0	505.25
Mean	332.00	476.75	692.25	541.50	
CD at 5%	C=8.77	D=8.77		C×D=17.53	

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value, however its effect was at par with that of 10^{-5}M GA_3 x 12 h and 10^{-5}M GA_3 x 16 h. Interaction 10^{-5}M GA_3 x 8 h gave 123.49% higher value than the lowest value giving interaction 0 M GA_3 x 4 h (Table 18).

4.3.3 1000-seed weight

Soaking treatment of 10^{-5}M GA_3 gave the maximum value, however its effect was at par with that of 10^{-3}M GA_3. Soaking with 10^{-5}M GA_3 gave 8.32% higher value than 0 M GA_3.

The effect of soaking durations on 1000-seed weight was not found significant.

The interaction effect on this parameter was also not found significant (Table 19).

4.3.4 Seed yield per plant

Soaking treatment 10^{-5}M GA_3 gave the maximum value. Its effect was followed by that of 10^{-3}M GA_3. Soaking with 10^{-5}M GA_3 gave 42.2% higher value than 0 M GA_3.

The effect of soaking durations on seed yield was not found significant.

Interaction of 10^{-5}M GA_3 x 8 h gave the maximum value, however its effect was at par with that of 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h and 10^{-5}M GA_3 x 4 h. Interaction 10^{-5}M GA_3 x 8 h gave 44.76% higher value than the lowest value giving interaction 0 M GA_3 x 4 h (Table 19).

4.3.5 Biological yield per plant

Soaking treatment 10^{-5}M GA_3 gave the maximum value, however its effect was at par with that of 10^{-3}M GA_3 and 10^{-7}M GA_3. Soaking with 10^{-5}M GA_3 gave 1.38% higher value than 0 M GA_3.

Table 19. **Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on 1000-seed weight and seed yield per plant of sunflower at harvest (Mean of four replicates)**

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
	1000-seed weight (g)				
4	46.60	47.72	50.26	49.98	48.64
8	47.87	48.79	51.58	50.17	49.60
12	47.15	48.67	51.42	49.35	49.15
16	46.84	47.80	50.91	48.58	48.53
Mean	47.12	48.25	51.04	49.52	
CD at 5%	C=2.54	D=NS		C×D=5.07	
	Seed yield per plant (g)				
4	19.57	22.15	28.03	25.49	23.81
8	20.01	22.59	28.33	25.76	24.17
12	19.93	22.47	28.29	25.64	24.08
16	19.79	22.29	28.16	25.58	23.95
Mean	19.83	22.38	28.20	25.62	
CD at 5%	C=1.27	D=NS		C×D=2.53	

NS = Non-significant

The effect of soaking durations was not found significant on this parameter.

The effect of interactions was also not found significant (Table 20).

4.3.6 Harvest index

Soaking treatment 10^{-5}M GA_3 gave the maximum value. Its effect was followed by that of 10^{-3}M GA_3. Soaking with 10^{-5}M GA_3 gave 40.28% higher value than 0 M GA_3.

The effect of soaking durations was not found significant on this parameter.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value, however its effect was at par with that of 10^{-3}M GA_3 x 12 h, 10^{-3}M GA_3 x 16 h, 10^{-3}M GA_3 x 4 h, 10^{-5}M GA_3 x 8 h, 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h and 10^{-5}M GA_3 x 4 h. Interaction 10^{-3}M GA_3 x 8 h gave 41.38% higher value than the lowest value giving interaction 0 M GA_3 x 4 h (Table 20).

4.3.7 Oil content

Soaking treatment 10^{-3}M GA_3 gave the maximum value, with its effect being at par with that of 10^{-5}M GA_3. Soaking with 10^{-3}M GA_3 gave 12.10% higher value than 0 M GA_3.

The effect of soaking durations was not found significant on this parameter.

Interaction 10^{-3}M GA_3 x 8 h gave the maximum value, however its effect was at par with that of 10^{-3}M GA_3 x 12 h, 10^{-3}M GA_3 x 16 h, 10^{-3}M GA_3 x 4h, 10^{-5}M GA_3 x 8 h, 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h and 10^{-5}M GA_3 x

Table 20. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on biological yield per plant and harvest index of sunflower at harvest (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
	Biological yield per plant (g)				
4	58.98	59.58	59.56	59.54	59.68
8	59.88	59.97	59.96	59.94	60.00
12	59.98	60.32	60.31	60.29	59.98
16	59.91	60.12	60.10	60.08	59.96
Mean	59.41	59.94	60.23	60.06	
CD at 5%	C=0.69	D=0.69		C×D=NS	
	Harvest index (%)				
4	33.18	36.98	46.74	42.55	39.86
8	33.59	37.67	46.91	42.84	40.25
12	33.46	37.48	46.90	42.65	40.12
16	33.25	37.18	47.71	42.57	39.93
Mean	33.37	37.33	46.81	42.65	
CD at 5%	C=1.71	D=NS		C×D=3.41	

NS = Non-significant

4 h. Interaction 10^{-3}M GA_3 x 8 h gave 14.93% higher value than the lowest value giving interaction 0 M GA_3 x 4 h (Table 21).

4.3.8 Oil yield per plant

Soaking treatment 10^{-5}M GA_3 gave the maximum value. Its effect was followed by that of 10^{-3}M GA_3. Soaking with 10^{-5}M GA_3 gave 55.06% higher value than 0 M GA_3.

Soaking for 8 h gave the maximum value, however its effect was at par with that of 12 h and 16 h soaking. Soaking for 8 h gave 3.72% higher value than 4 h soaking.

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value, however its effect was at par with that of 10^{-5}M GA_3 12 h, 10^{-5}M GA_3 x 16 h and 10^{-5}M GA_3 x 4 h. Interaction 10^{-5}M GA_3 x 8 h gave 63.34% higher oil yield than the lowest value giving interaction 0 M GA_3 x 4 h (Table 21).

4.4 Oil quality characteristics

The effect of GA_3 soaking concentrations and durations alone or in combination on acid value and interaction effect on saponification value was found to be significant only (Tables 22-23).

4.4.1 Acid value

Soaking treatment 10^{-5}M GA_3 gave the maximum value. Its effect was followed by that of 10^{-3}M GA_3 and 10^{-7}M GA_3. Soaking with 10^{-5}M GA_3 gave 72.88% higher value than 0 M GA_3.

Soaking for 8 h proved best, however its effect was at par with that of 12 h soaking. Soaking for 8 h gave 20.00% higher value than 4 h soaking.

Table 21. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on oil content and oil yield per plant of sunflower at harvest (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
			Oil content (%)		
4	22.10	23.30	24.20	25.00	23.65
8	22.80	23.70	24.90	25.40	24.20
12	22.60	23.50	24.60	25.30	24.00
16	22.40	23.40	24.30	25.10	23.80
Mean	22.48	23.47	24.50	25.20	
CD at 5%	C=0.82	D=NS		CxD=1.63	
			Oil yield per plant (g)		
4	4.31	5.51	6.78	6.37	5.65
8	4.55	5.34	7.04	6.53	5.86
12	4.50	5.27	6.95	6.48	5.80
16	4.43	5.21	6.83	6.41	5.72
Mean	4.45	5.24	6.90	6.44	
CD at 5%	C=0.19	D=0.19		CxD=0.39	

NS = Non-significant

Interaction 10^{-5}M GA_3 x 8 h gave the maximum value. However, its effect was at par with that of 10^{-5}M GA_3 x 12 h, 10^{-5}M GA_3 x 16 h, 10^{-5}M GA_3 x 4 h, 10^{-3}M GA_3 x 8 h and 10^{-3}M GA_3 x 12 h. Interaction 10^{-5}M GA_3 x 8 h gave 111.76% higher value than the lowest value giving interaction 0 M GA_3 x 4 h (Table 22).

4.4.2 Iodine value

The effect of soaking concentrations and soaking durations as well as their interactions was not found significant on this parameter (Table 22).

4.4.3 Saponfication value

The effect of soaking concentrations was not found significant on this parameter.

The effect of soaking durations was also not found significant on this parameter.

Interaction 10^{-3}M GA_3 x 8 h gave the maximum value. However, its effect was at par with that of 10^{-3}M GA_3 x 12 h, 10^{-3}M GA_3 x 16 h, 10^{-3}M GA_3 x 4 h, 10^{-5}M GA_3 x 8 h, 10^{-5}M GA_3 x 12 h and 10^{-5}M GA_3 x 16 h. Interaction 10^{-3}M GA_3 x 8 h gave 1.64% higher value than the lowest value giving interaction 0 M GA_3 x 4 h (Table 23).

Table 22. **Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on acid value and iodine value of the oil of sunflower at harvest (Mean of four replicates)**

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
	Acid value (mg KOH/g soil)				
4	0.51	0.72	0.97	0.78	0.75
8	0.69	0.87	1.08	0.97	0.90
12	0.62	0.84	1.05	0.94	0.86
16	0.55	0.76	0.99	0.85	0.79
Mean	0.59	0.80	1.02	0.88	
CD at 5%	C=0.08	D=0.08		C×D=0.15	
	Iodine value (g I/100 g oil)				
4	120.55	121.18	122.14	123.90	121.74
8	120.93	121.85	122.80	123.72	122.32
12	120.90	121.82	122.77	123.41	122.22
16	120.87	121.50	122.45	123.12	121.98
Mean	120.81	121.58	122.54	123.33	
CD at 5%	C=NS	D=NS		C×D=NS	

NS = Non-significant

Table 23. Effect of concentrations (C) and soaking durations (D) of pre-sowing seed treatment of GA_3 on saponification value of the oil of sunflower at harvest (Mean of four replicates)

Soaking durations (h)	Soaking concentrations (M GA_3)				Mean
	0	10^{-7}	10^{-5}	10^{-3}	
	Saponification value (mg KOH/g oil)				
4	188.63	190.03	190.52	191.44	190.15
8	189.33	190.45	190.74	191.72	190.56
12	189.26	190.31	190.66	191.65	190.47
16	189.19	190.17	190.59	191.58	190.38
Mean	189.10	190.24	190.62	191.59	
CD at 5%	C=NS	D=NS		CxD=1.17	

NS = Non-significant

Discussion

CONTENTS

CHAPTER 5

DISCUSSION

Plant growth and development are controlled by various intrinsic and extrinsic factors. Phytohormones belong to the former. They get involved through the modification of transcription, translation and/or differential sensitivity of the tissue. GA_3 is one of the growth promoting phytohormones. It has been shown to regulate many facets of plant life, including seed *germination, vegetative growth and differentiation* (De-La-Guardia and Benlloch, 1980; Ray and Choudhuri, 1981; Bangal *et al.*, 1982; Simpson *et al.*, 1982; Erdel and Dhakal, 1988; Singh and Sahu, 1993; Agrawal *et al.*, 1994; Khan *et al.*, 1996, 2002, 2006; Azam, 2003; Khan and Samiullah, 2003; Siddiqui and Mohammad, 2003; Afroz *et al.*, 2005; Khan, 2008; Siddiqui *et al.*, 2008).

Keeping its role in growth and development in view, a factorial randomized pot experiment was performed on sunflower cv. PAC 3776 under the agroclimatic conditions of Aligarh, with levels of GA_3 being one variant and soaking durations the other. As mentioned earlier, the performance of the crop was assessed in terms of growth characters, physiological and bio-chemical parameters as also yield and quality characteristics. The results are discussed below, parameter-wise, in the light of the knowledge of the subject and research work carried out by other workers.

5.1 Effect of pre-sowing seed treatment

The observed ameliorative effect of pre-sowing seed treatment with GA_3 particularly at 10^{-5}M GA_3 over the water-soaked treatment (control) on shoot

length per plant and leaf area per plant at 50 and 70 DAS (Tables 4 and 6) can be traced to its various roles in plants. For example, treatment with GA_3 enhances, among other processes, absorption of nutrients (Balki and Padole, 1982; Singh *et al.*, 2005), activity of enzymes (Khan, 1996; Chanda *et al.*, 1998; Yuan and Xu, 2001; Afroz *et al.*, 2005), cell division (Liu and Loy, 1976; Moore, 1989; Huttly and Phillips, 1995; Arteca, 1996), cell enlargement and differentiation (Huttly and Phillips, 1995; Mobin, 1999; Buchanan *et al.*, 2000; Marschner, 2002), chlorophyll content (Afroz *et al.*, 2005), deoxyribose nucleic acid, ribose nucleic acid and protein synthesis (Broughton 1968; Johri and Varner, 1968; Roth-Benjerano and Lips, 1970; Pain and Dutta, 1977; Mozer, 1980), activity of ribulose bisphosphate carboxylase, a key enzyme controlling photosynthetic carbon fixation of plants (Yuan and Xu, 2001), synthesis of other enzymes, especially hydrolases (Marschner, 2002), membrane permeability (Wood and Paleg, 1972, 1974; Crozier and Turnbull, 1984), elongation of internode (Krishnamoorthy, 1981; Kumar *et al.*, 1996), metabolism of storage products (Mobin, 1999), N use efficiency (Khan *et al.*, 2002), ribose and polyribose multiplication (Evins and Varner, 1972), synthesis of new materials (Mobin, 1999) and transport of photosynthates (Mulligan and Patrick, 1979; Aloni *et al.*, 1986; Daie *et al.*, 1986; Estruch *et al.*, 1989; Hayat *et al.*, 2001). Thus, GA_3 treatment might have affected directly or indirectly the two parameters of treated plants. These results broadly corroborate the findings of Khan *et al.* (2003) on sunflower and Saran and Mehta (1985), Saran *et al.* (1992), Khan and Samiullah (2003) and Siddiqui *et al.* (2008) on mustard. The enhancement in shoot length and leaf area (Tables 4 and 6) was expectedly reflected in increased fresh and dry weight of treated plants (Tables 8 and 10). Correlation studies also reveal that, at 50 and 70 DAs respectively, fresh weight

has relationship with shoot length (r = 0.988 and 0.906) and leaf area (r = 0.987 and 0.915) and dry weight with shoot length (r = 0.923 and 0.981) and leaf area (r = 0.917 and 0.980). Similar increase in dry matter production of sunflower due to application of pre-sowing seed treatment with GA_3 has also been reported by Khan *et al.* (2003) and Siddiqui and Mohammad (2003).

The enhancement in leaf chlorophyll content, CA activity and NR activity at both stages (50 and 70 DAS) resulting from pre-sowing seed treatment with GA_3 particularly at 10^{-5}M GA_3 over the control (Tables 12-14) is worthmentioning. The improvement in leaf chlorophyll content due to GA_3 treatment may be ascribed to its roles in various metabolic processes related to chlorophyll content. The enhancing effect of GA_3 on CA and NR activities may be attributed to the hormone-induced increase in transcription and/or translation of the genes that code for CA (Okabe *et al.*, 1980, 1984; Sugiharto *et al.*, 1992) and NR (Roth-Benjerano and Lips, 1970; Ahmad, 1988, 1994; Ahmad and Hayat, 1999). These results corroborate the findings of Siddiqui and Mohammad (2003) on sunflower and Saran *et al.* (1992), Khan and Samiullah (2003) and Afroz (2006) on mustard.

The improvement in leaf N, P and K contents at both stages (50 and 70 DAS) resulting from pre-sowing seed treatment with GA_3 particularly at 10^{-3}M GA_3 over the control (Tables 15 to 17) is not far to seek. The increase in the nutrient content may be related to the role of GA_3 in enhancing the permeability of membranes and absorption of nutrients (Balki and Padole, 1982; Singh *et al.*, 2005; Wood and Paleg, 1972, 1974; Crozier and Turnbull, 1984).

The enhancement in head diameter, seeds per head and 1000-seed weight resulting from pre-sowing seed treatment with GA_3 particularly at 10^{-5}M GA_3 over the control (Tables 18 and 19) is understandable. Vegetative growth of a crop and physio-biochemical processes control the number and size of photosynthesizing sites responsible for production of photosynthates even after flowering and their partitioning ultimately controls yield characteristics (Prasad *et al.*, 1978; Yoshida, 1981; Khan, 2008). It may also be added that exogenous application of GA_3 promotes differentiation leading to enhanced number of flowers (Hutty and Phillips, 1995; Mobin, 1999; Buchanan *et al.*, 2000; Marschner, 2002). Its treatment may also be helpful in the desirable development of under-developed seeds particularly at the centre of the head as GA_3 also causes cell division and cell enlargement (Liu and Loy, 1976; Moore, 1989; Huttly and Phillips, 1995, Arteca, 1996; Marschner, 2002). These roles of GA_3 directly or indirectly may be responsible for an increase in number of flowers coupled with the desirable development of under-developed seeds that result in higher values for head diameter and seeds per head of treated plants (Table 18). Also, its promoting effect on net photosynthetic rate (Afroz *et al.*, 2005), membrane permeability (Wood and Paleg, 1972, 1974; Crozier and Turnbull, 1984) and transport of photosynthates (Mulligan and Patrick, 1979; Aloni *et al.*, 1986; Daie *et al.*, 1986; Estruch *et al.*, 1989; Hayat *et al.*, 2001) may be helpful in favouring the partitioning of photosynthates towards developing seeds in the head, hence higher value for 1000-seed weight of treated plants (Table 19). Thus, the higher values for vegetative, physio-biochemical and yield characters of treated plants (Tables 18 to 21) may culminate in higher seed yield (Table 19). This proposition is further confirmed by correlation studies wherein seed yield has shown positive relationship with

the various parameters studied for example, at 50 and 70 DAS respectively, leaf area (r = 0.984 and 0.965), dry weight (r = 0.970 and 0.990), CA activity (r = 0.943 and 1.000), chlorophyll content (r = 0.978 and 0.201), leaf N content (r = 0.884 and 0.809), leaf P content (r = 0.921 and 0.872) and at harvest head diameter (r = 0.728), seeds per head (r = 0.982) and 1000-seed weight (r = 0.996). These results are also in accordance with the findings of Khan *et al.* (2003) and Siddiqui and Mohammad (2003).

5.2 Effect of soaking duration

The observed enhancement in the values for most of the growth characters, physiological and biochemical parameters and yield and quality characteristics at the various growth stages (Tables 4-23) resulting from pre-sowing seed treatment (with GA_3) for 8 h over 4 h is noteworthy. It may be added here that a specific concentration of a phytohormone like other metabolites is required for optimum performance of a plant. Pre-sowing seed treatment with GA_3 for 4 h may not be sufficient for accumulation of the hormone inside the seeds at the specific level as the seed coat of sunflower is thick and hard. The specific level of the hormone might have been achieved by soaking the seeds for 8 h, hence higher values for most parameters studied. These results corroborate the finding of Shah (2007) who also reported the effect of pre-soaking duration on the performance of black cumin.

5.3 Conclusions

The present study revealed the following:

1. Soaking of seeds in GA_3 was more effective than the water soaked control for most of the parameters studied.

2. The optimum concentration obtained for soaking the seeds in GA_3 was 10^{-5}M.

3. Duration of pre-soaking seed treatment with GA_3 was also observed to be effective.

4. Soaking the seeds in GA_3 for 8 h was found to be optimum.

5. Finally, it may be concluded that soaking the seeds with 10^{-5}M GA_3 for 8 h is best for growth and development of sunflower cv. PAC 3776.

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Appendix

APPENDIX

Acetone (80%)

80 ml acetone was mixed with 20 ml DDW.

Aminonaphthol sulphonic acid

500 mg 1-amino-2-naphthol-4-sulphonic acid was dissolved in 195 ml 15% sodium bisulphite to which 5 ml 20% sodium sulphite solution was added. The solution was kept in an amber coloured bottle.

Bromothymol blue indicator in ethanol (0.002%)

0.002 g bromothymol blue was dissolved in approximately 100 ml ethanol.

Cystein hydrochloride solution (0.2 M)

48 g cystein hydrochloride was dissolved in the sufficient volume of DDW and the final volume was made up to 100 ml using DDW.

GA_3 stock solution (10^{-3}M)

34.637 g GA_3 was dissolved in the sufficient volume of ethanol in 100 ml volumetric flask. The final volume was made up to the mark using DDW. The required concentrations (10^{-7} and 10^{-5}) were prepared by diluting the stock solution using DDW.

Hydrochloride acid (0.05 N)

4.3 ml hydrochloric acid was mixed with 95.7 ml DDW to get 100 ml 0.05N HCl.

Hydrochloric acid (0.5 N)

21.49 ml hydrochloric acid was mixed with 478.51 ml DDW to get 500 ml 0.5 N HCl.

Iodine monochloride solution

13 g iodine was dissolved in a mixture of 300 ml carbon tetrachloride and 700 ml glacial acetic acid and the resulting solution was divided into solution A and B. To 20 ml of solution A, 15 ml potassium iodide

solution and 100 ml DDW were added and the mixture was titrated against 0.1 N sodium thiosulphate solution, using starch solution as an indicator. Chlorine gas was passed through solution B until the amount of the sodium thiosulphate solution required for the titration was not more than double of that needed in solution A.

Isopropanol solution (5%)

5 ml isopropanol was mixed with 95 ml of DDW.

Mercuric chloride solution (0.1%)

100 mg $HgCl_2$ was dissolved in the sufficient volume of DDW in 100 ml volumetric flask and the final volume was made using DDW.

Molybdic acid reagent

6.25 g ammonium molybdate was dissolved in 175 ml 10 N H_2SO_4.

Naphthylethylenediamine dihydrochloride (NED-HCl) solution (0.02%)

20 mg naphthylenediamine dihydrochloride was dissolved in the sufficient volume of DDW and the final volume was made up to 100 ml using DDW.

Nessler's reagent

3.5 g potassium iodide was dissolved in 100 ml DDW to which 4% mercuric chloride was added with stirring until a slide red precipitate remains, then 120 g NaOH was mixed with 250 ml DDW. The mixture was kept in an amber coloured bottle.

Phenolphthalein solution (1%)

1 g phenolphthalein was dissolved in the sufficient volume of 95% ethanol and the volume was made up to 100 ml using the ethanol.

Phosphate buffer (0.2 M) for pH 6.8

(a) 27.80 g sodium dihydrogen ortho-phosphate was dissolved in the sufficient volume of DDW and the final volume was made up to 1000 ml.

(b) 53.65 g disodium hydrogen ortho-phosphate was dissolved in the sufficient volume of DDW and the final volume was made up to 1000 ml.

51 ml solution (a) was mixed with 49 ml solution (b).

Phosphate buffer (0.1 M) for pH 7.5

(a) 13.6 g potassium dihdyrogen orthophosphate was dissolved in the sufficient volume of DDW and the final volume was made up to 1000 ml using DDW.

(b) 17.42 g dipotassium hydrogen orthophosphate was dissolved in the sufficient volume of DDW and final volume was maintained up to 1000 ml using DDW.

160 ml solution (a) was mixed with 840 ml solution (b)

Potassium hydroxide solution (0.1 N KOH)

5.6 g potassium hydroxide was dissolved in the sufficient volume of 95% ethanol and the final volume was made up to 1 litre using the ethanol.

Potassium hydroxide solution (0.5 N KOH)

28 g potassium hydroxide was dissolved in the sufficient volume of 95% ethanol and the final volume was made up to 1 litre using the ethanol.

Potassium iodide solution

150 g potassium iodide was dissolved in the sufficient volume of DDW and the final volume was made up to 1 litre using DDW.

Potassium nitrate solution (0.02 M)

2.02 g potassium nitrate was dissolved in the sufficient volume of DDW and final volume was maintained up to 1000 ml with DDW.

Sodium bicarbonate solution (0.2 M) in 0.02 M sodium hydroxide solution

16.8 g sodium bicarbonate was dissolved in 0.02 M sodium hydroxide solution (0.8 g NaOH/1) and the final volume was made up to 1000 ml using the sodium hydroxide solution.

Sodium hydroxide solution (2.5 N)

100 g sodium hydroxide was dissolved in the sufficient volume of DDW and the final volume was maintained up to 1000 ml using DDW.

Sodium silicate solution (10%)

10 g sodium silicate was dissolved in the sufficient volume of DDW and the final volume was made up to 100 ml using DDW.

Sodium thiosulphate solution (0.1 N)

24.8 g sodium thiosulphte was dissolved in the sufficient volume of DDW and the final volume was made up to 1 litre using DDW.

Solvent mixture

Ethanol (95%) was mixed with diethyl ether in 1:1 ratio. This mixture of solvents was neutralized just before use by means of 0.1 N potassium hydroxide solution in the presence of phenolphthalein solution as an indicator.

Starch solution (1%)

1 g soluble starch was dissolved in the sufficient volume of DDW and the final volume was made up to 100 ml using DDW.

Sulphanilamide solution (1%)

1 g sulphanilamide was dissolved in the sufficient volume of 3 N hydrochloric acid and the final volume was made up to 100 ml using 3 N hydrochloric acid.

Sulphuric acid (10 N)

27.2 ml sulphuric acid was mixed with 72.8 ml DDW to get 100 ml 10 N H_2SO_4.